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Cotton Research and Development Corporation

FINAL REPORT 2016

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Part 1 - Summary Details

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

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Part 3 – Final Report

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

Background

1. Outline the background to the project.

Bemisia tabaci (Gennadius), Middle East – Asia Minor 1 (MEAM1) or B-biotype, commonly referred to as silverleaf whitefly (SLW) is an introduced pest of Australian agriculture, particularly cotton and horticulture.

History and pest status of SLW

Bemisia tabaci (MEAM1) were introduced into Australia in 1994, and has since established throughout the regions where cotton and horticultural crops are grown.

Contamination of cotton lint through the production of sticky honeydew is the primary reason SLW are considered a pest of cotton. Sticky honeydew can cause serious problems during processing and is considered a quality control problem with penalties applied to producers of sticky lint.

Sustainable management of SLW is a major challenge due to their capacity to develop resistance to insecticides, including insect growth regulators like pyriproxyfen (Admiral®).

The first major pest outbreak of SLW occurred at Emerald in 2001–2002. Since then most cotton production regions have had periodic outbreaks. These have been largely controlled through the use of registered chemistry, with heavy reliance on pyriproxyfen.

Since the first outbreak of SLW in early 2000s, there has been a steady increase in the number of insecticides registered for control. Currently there are seven available products, with another likely to be registered for the 2016/17 season.

To improve biological control of SLW, a parasitoid wasp, *Eretmocerus hayati* Zolnerowich and Rose was introduced into Australia in 2004 and was widely released in NSW and QLD. This wasp is now well established and is helping to suppress SLW population growth.

Resistance monitoring and management

Silverleaf whitefly has developed resistance to a range of insecticides including; carbamates (1A), organophosphates (1B), pyrethroids (3A), neonicotinoids (4A), pyriproxyfen (7C), buprofezin (16) and amitraz (19).

An insecticide resistance management strategy (IRMS) has been developed for SLW control in cotton. The strategy revolves around windowing of products and restricting the number of insecticide applications within a mode of action group per season to slow the selection of resistant genotypes.

To assist with management, the decision support tool ‘SLW threshold matrix’ provides advice on product selection for a given whitefly population and crop development stage. The matrix is designed to help with timing of sprays, particularly IGRs, so SLW populations are controlled prior to when the crop is most susceptible to damage.

Since the early 2000s, resistance levels of SLW have been monitored by collecting and screening populations against registered chemistry. This data is useful for informing industry of emerging resistance issues and to formulate management strategies.

Monitoring has continued in this project, with testing of whitely populations and reporting on resistance to registered chemistry, as well as development of baseline susceptibility data on newly registered insecticides. The project continues to provide diagnostic services including screening samples for exotic species in collaboration with Dr Sharon van Brunschot (UQ).

Toxicity of insecticides on *Eretmocerus hayati*

The introduced parasitoid *E. hayati* has made a valuable contribution to the biological control of SLW, but like other natural enemies it can be adversely affected by insecticides used to control whitefly and other pests in cotton.

There is published information on the effect of some (generally older) insecticides on *E. hayati*, but for newer insecticide only limited data is available. Our aim was to test the toxicity of recently registered insecticides and to update the cotton pest management guide with this data.

Biological control and predator studies

The ecosystem service of biological control is an important component of integrated pest management (IPM) in cotton. Fundamental to improving the use of biological control in IPM is knowledge of predator biology and evaluating their potential contribution in pest control. Over several projects, the contribution of several important natural enemies of cotton aphid, *Aphis gossypii* Glover and SLW has been studied. This includes the ladybeetle, *Hippodamia variegata* Goeze, the big-eyed bug *Geocoris lubra* Kirkaldy, damsel bugs *Nabis kinbergii* Reuter and the wasp parasitoid *Lysiphlebus testaceipes* (Cresson).

This project studied development, prey consumption and prey choice in three common cotton predators: minute two-spotted ladybird *Diomus notescens* Blackburn, transverse ladybird *Coccinella transversalis* Fabricius and green lacewing *Mallada signatus* Schneider.

Objectives

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

The project had four major objectives:

1. Identify whitefly species/biotype
2. Test whitefly for insecticide resistance
3. Examine insecticide impacts on the parasitoid *E. hayati*
4. Investigate predator preferences and consumption

The aim of the first objective was to provide both a diagnostic service to industry by identifying whitefly samples, and support biosecurity surveillance. Requests for species identification were minimal, and mainly from areas where SLW are not a common problem (i.e. Darling Downs). All field collection used to establish populations for resistance testing were subsampled and sent to Dr Sharon van Brunschot for molecular identification of biotype. In total 123 individuals from 46 sites were tested. Other biotypes of *B. tabaci*,

particularly Mediterranean (Q biotype) pose a significant biosecurity risk to Australian agriculture so early detection is of value.

Testing SLW for resistance to insecticides was the core objective of this project. Testing of SLW resistance levels to registered and high use insecticides was completed for three cotton production seasons. Representative SLW populations were collected from several cotton production regions, especially those that regularly experience high SLW pressure. Testing results were annually presented at the transgenic and insect management strategies (TIMS) technical panel meetings. Aligned with this milestone has been the collection of baseline susceptibility data on new insecticides undergoing registration, including testing against lab susceptible and resistant strains.

Insecticide impacts on the SLW parasitoid, *E. hayati* were studied using lab and glasshouse experiments. In total we investigated the toxicity of eight insecticides and used data from these experiments to update the cotton pest management guide.

The predator studies focused on the development, prey consumption and prey choice behaviour of three predators of cotton aphid, *A. gossypii.* The predators studied were minute two-spotted ladybird *D. notescens*, transverse ladybird *C. transversalis* and green lacewing *M. signatus*. In 2016, research on the minute two-spotted ladybird was published in the journal 'Biological Control' (96: 101–107).

Methods

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

A general methodology is outlined for each milestone. Methods used in this project have been developed from three sources; the Insecticide Resistance Action Committee, journal articles, and from advice of other researchers e.g. Arizona study tour in a previous project (03DAQ006).

1.1 Provide diagnostic service for industry including sampling for exotic species

Whitefly collected as part of our regional collecting for resistance testing were subsampled and sent to Dr Sharon van Brunschot at the University of Queensland for molecular identification to species level.

DNA was extracted from individual adult whiteflies using a modified version of the high-throughput silica spin column plate method developed by Dr James Hereward (UQ). The mitochondrial COI gene of whitefly samples were amplified using the PCR primers C1-J-2195/ TL2-N-3014, using optimized PCR conditions. Amplicons were subjected to conventional Sanger sequencing by Macrogen. Pairwise comparisons were performed using the MUSCLE algorithm implemented in Geneious v7, with manual checking and editing.

Representative sequences of the global genetic diversity of *Bemisia tabaci* documented in the current literature were used for phylogenetic reconstruction. Sequences were aligned using MUSCLE. The best-fit model of evolution was determined from likelihood ratio tests performed using jModelTest. Phylogenetic relationships were reconstructed via Bayesian

analyses using MRBAYES v3.1.2 implemented in Geneious v7. Sequences from close relatives of these taxa were included as outgroups and for rooting the phylogenetic tree.

2.1 Collect and maintain whitefly colonies from cotton regions

With the cooperation of local growers and agronomists, whitefly collections were made from cotton farms in regions where whitefly are a pest, including Emerald, Theodore, Darling Downs, St George, Goondiwindi, Boggabilla, Moree, Wee Waa, and Hillston.

Whitefly collections were made using a Stihl blower/vac running on idle, with a modified collection net placed over the entrance of the suction port. Collected material was transferred onto a caged cotton plant in the field. As these collections contain other insects, upon returning to the lab, adults whitefly were aspirated from these collections, transferred onto a cotton plant and used to establish a population (F1 generation), that was then kept in the glasshouse.

Some strains were provided by agronomists as either nymphs or adults on collected leaves. The leaves were transferred into an emergence box, so nymph development could be completed and as adults they could be collected to start glasshouse strains.

Each whitefly strain was maintained as a series of discrete generations. Adults laid eggs over a short period of 3 to 4 days before being removed. The juveniles then developed through and were used both in bioassays and to found the next generation.

Whitefly strains resistant to pyriproxyfen and bifenthrin were kept in the glasshouse and routinely pressured to maintain high level resistance for the duration of the project. A strain initially collected from Gatton with resistance to clothianidin was replaced with a population collected at Griffith. Since collection the Griffith strain has been routinely pressured to maintain resistance to clothianidin. A susceptible strain with very limited exposure to insecticides has been maintained for the duration of the project. The cotton variety, Sicot 71BRF was used for rearing of whitefly and in resistance testing bioassays.

2.2 Test whitefly for resistance

Bemisia tabaci were tested for resistance to insecticides registered for whitefly control as well as the clothianidin (high use insecticide) and sulfoxaflor (newly registered insecticide) (Table 1). Bioassays used were based on those developed in prior projects; 03DAQ006 and DAQ1104.

<u>Pyriproxyfen</u>

To test resistance levels to pyriproxyfen, a leaf dip assay was used. Eggs were laid on leaves overnight prior to being treated. Doses tested ranged from 0.001 to 10 ppm usually at log or half log increments. Egg mortality was assessed at 10 days post treatment. Five treatments and a control were used with five replicates; each replicate contained around 30 individuals.

Table 1. Summary of bioassay methodology used for each insecticide.

Insecticide	Trade name	Conc.	Dose Range (ppm)	Bioassay type	Development stage targeted	Duration (days)
Pyriproxyfen	Admiral	100 g/L	0.001 - 10	leaf dip	eggs	10
Diafenthiuron	Pegasus	500 g/L	3 - 300	leaf dip	adults	3
Cyantraniliprole	Exirel	100 g/L	0.006 - 1	systemic uptake	1st instar nymphs	13
Spirotetramat	Movento	240 g/L	1 - 300	leaf dip	2nd instar nymphs	11
Bifenthrin	Talstar	250 g/L	1 - 1000	leaf dip	adults	2
Dinotefuran	Starkle	200 g/kg	0.1 - 320	leaf dip & systemic uptake	adults	3
Clothianidin	Shield	200 g/L	0.1 - 10000	leaf dip & systemic uptake	adults	3
Sulfoxaflor	Transform	240 g/L	0.1 - 1000	leaf dip	adults	3

Diafenthiuron

Resistance to diafenthiuron was tested using adult whitefly with a leaf dip assay. Adults were enclosed in clip cages on the treated leaves and their survival recorded at three days post treatment. Five treatments and a control were used, with five replicates. For each replicate 15–20 individuals were tested. Female adults were used for adult bioassays as they are diploid, while males are haploid. This methodology (with variations outlined below) has been used for all adult whitefly – leaf dip assays.

Cyantraniliprole

The bioassay used for cyantraniliprole was a systemic uptake assay and mortality assessment was based on 2nd instar survival. In 2013/14 and 2014/15, seedling plants were used and the methodology developed by DuPont was adopted. After experiencing moderate levels of seedling death during assays, the methodology was adjusted to use the first or 2nd true leaf of seedlings. This method was used for the 2015/16 bioassays.

Spirotetramat

To test for spirotetramat resistance, a leaf dip assay was used. At the time of treatment leaves had 2nd instar nymphs and survival was assessed at 11 days post treatment. Five treatments and a control were replicated five times, with around 30 individuals per replicate. For the 2013/14 and 2014/15 seasons all field collected populations were tested. In 2016 only one population per region was tested. This decision was based on the increased number of insecticides being tested in the project (with the registration of Starkle) and the limited use of spirotetramat by the industry.

Bifenthrin

An adult whitefly – leaf dip assay (as described above) was used for bifenthrin but with mortality assessed at 2 days.

Dinotefuran

Dinotefuran is a newly registered product for whitefly and mirid control in cotton. In 2014/15 we collected baseline susceptibility data for this insecticide. Two methods were tested: a systemic uptake and leaf dip assay. With the systemic method, leaves with petiole attached were used. To uptake the insecticide, the petiole was placed in a solution of dinotefuran for 24hrs. After 24hrs the leaves were transferred into vials containing distilled water. Whitefly adults were then enclosed in clip cages on the treated leaves and mortality was assessed after 3 days. The foliar method used the adult whitefly leaf dip assay as used for diafenthiuron. For the baseline studies, 7 treatments and a control were tested with 5 replicates of 20 female adults. The leaf dip method was adopted for testing the 2015/16 collections as it was most reflective of field application, but with 5 treatments and a control.

Clothianidin

Resistance to clothianidin was tested using an adult whitefly – leaf dip assay. In 2014/15 a systemic assay adapted from the dinotefuran systemic assay was trialled. For the 2015/16 collections, testing was reduced to one population per region and a leaf dip assay was used with an upper dose of 10000 ppm.

Sulfoxaflor

Resistance to sulfoxaflor was tested using an adult whitefly – leaf dip assay. Testing was conducted in 2013/14 and 2014/15.

3.1 Test toxicity of insecticides on whitefly parasitoid

Eretmocerus hayati were obtained from a commercial insectary (Bugs for Bugs) and stored in an incubator prior to use in two experiments, where both direct and residual exposure was tested.

In experiment 1, the wasps used in the direct exposure experiment were transferred into cylindrical plastic containers (15 cm with 9 cm diameter) with gauze lids, fed a dilute (25%) honey solution and kept at 20°C for 4 days. The wasps used in the residue exposure experiments were left in the vials they were shipped in and stored in an incubator at 25°C for 24 to 48 hours prior to use. In experiment 2, wasps used in all treatments were transferred direct from the vials they were shipped in and were kept in a constant temperature room at 25 °C prior to use.

Cotton plants (Sicot 71) were grown in large pots (3 plants per pot) under glasshouse conditions for 8 weeks prior to use in the experiment.

Spray procedure

Treatments were applied with 3 x DG110015 nozzles and calibrated to deliver 106 L/ha based on 50 cm nozzle spacing and 5.0 km/hr walking speed. In experiment 1, flow was based on calibration of 440 mL/min/nozzle (1.8 bar gauge pressure) and in experiment 2, flow was based on calibration of 448 mL/min/nozzle (1.6 bar gauge pressure).

Testing focused on products where no information was available; however some of products with known toxicity were included as standards.

Insecticides tested:

1. Sulfoxaflor (Transform 240SC) at 300 mL/ha
2. Flonicamid (Mainman 500 WG) at 140 g/ha
3. Clothianidin (Shield 200g/L) at 250 mL/ha + Maxx at 2 mL/L
4. Fipronil (Maestro 200SC) at 125 mL/ha
5. Spirotetramat (Movento 240SC) at 400 mL/ha + Hasten at 1000 mL/100L
6. Cyantraniliprole (Exirel 100SE) at 600 mL/ha + Hasten at 500 mL/100L
7. Dinotefuran (Starkle 200SG) at 90 g/ha
8. Dinotefuran (Starkle 200SG) at 375 g/ha
9. Bifenthrin (Talstar 250EC) at 320 mL/ha

After treatment, plants were returned to the glasshouse and kept in shallow trays filled with water.

Experimental procedure

1. Direct exposure

To test the effect of direct exposure of insecticides on the parasitoid, wasps were placed into cylindrical gauze cages (94 x 46 mm). For each treatment, five replicates were tested with each replicate containing 20 wasps. Cages were placed 50 cm off the ground and sprayed with a boom spray. After exposure cages were taken to the lab and (with the aid of a microscope) wasps were assessed as alive, dead or moribund after 30 minutes.

2. Residue exposure

To test the toxicity of insecticide residues, wasps were confined in ventilated glass bioassay cylinders (15 cm long x 4 cm diameter) with sections of treated leaf. One end of the cylinder was covered with fine gauze, and the other end attached to a ventilation system, which pumped air (0.5 m/sec) through the cylinder to minimise fumigation effects. A section of leaf material that had been sprayed with one of the insecticide treatments was added to each cylinder. Wasps were then added to the cylinder. Residues were tested at three intervals after the treatments had been applied (1, 2 and 8 days). Parasitoids were exposed to the residues for 4 hours and then assessed as alive or dead. For each treatment five replicates were tested and in each replicate 10 to 20 wasps were exposed.

4.1 Prey consumption studies

Rates of prey consumption and development of three natural enemies of cotton aphid (*A. gossypii*) were studied. The predators chosen for this study were:

- Minute two-spotted ladybird beetle – *Diomus notescens*
- Transverse ladybird beetle – *Coccinella transversalis*
- Green lacewing – *Mallada signatus.*

These three species where chosen based on four criteria:

- Abundance in cotton (based on ranking of abundance in 'The seasonal abundance and impact of predatory arthropods on *Helicoverpa* species in Australian cotton fields' J. Stanley PhD 1997)
- Lack of existing knowledge on their biology
- Predator status regarding *Bemisia tabaci* unknown
- Availability over the course of the project.

Adult predators were collected in the field, or in the case of green lacewing purchased from a commercial insectary (Bugs for Bugs) and then subsequently reared in the glasshouse on cotton aphid. Experiments were started by placing adults into plastic containers kept in a constant temperature room at 25 ± 3°C, 60± 12% RH and 15:10 L:D. These adult populations were fed daily and containers checked daily for eggs. When sufficient eggs were available, experiments were started. Eggs were stored in Petri dishes (8 cm diameter) without vents.

Egg development was monitored daily and more frequently after first emergence was observed.

Newly emerged larvae (<12 hours old) were carefully moved into individual experimental arenas, consisting of a cotton leaf with prey kept in an unvented Petri dish (8 cm diameter). To keep the leaf fresh, a moist filter paper (Whatman® 1) was used to line the bottom of the dish. Petri dishes were kept in a constant temperature room at 25 ± 3°C, 60± 12% RH and 14:10 L:D. The developmental instar of each larva and number of prey consumed was recorded daily, and prey was added to replace those eaten. Every second day, filter paper, leaves and prey were replaced to keep the development stage of the prey consistent.

Type of prey, its developmental stage and numbers offered varied between experiments, largely depending on the predator species and its developmental stage (Table 2.)

<u>Adult minute two-spotted ladybird – consumption, fecundity and lifespan</u>

Prey consumption of adult *D. notescens* was measured using the same experimental arena described for juveniles. The number of aphids provided daily was 75 nymphs (2^{nd} or 3^{rd} instar). Five controls were set up to estimate aphid mortality in the absence of the ladybeetle predator. Number of aphids eaten and number of eggs laid daily was recorded. Prey consumption was recorded for 54 days; fecundity and lifespan were recorded till the last individual died. Beetles were kept together for several hours after emergence to mate before being used in the experiment and beetles were again paired together after 40 days to mate.

Table 2. Species, developmental stage and number of prey offered in experiments for each predator larval stage.

Predator			Prey		
Species	**Development**	**Replicates**	**Species**	**Stage**	**Number provided**
Minute two-spot ladybird	all larval stages	40	cotton aphid	2nd	30
Transverse ladybird	1st & 2nd instar larva	40	cotton aphid	2nd/3rd	30
	3rd & 4th instar larva	30	cotton aphid	3rd/4th	60
Green lacewing	all larval stages	15	cotton aphid	3rd/4th	100
	all larval stages	15	silverleaf whitefly	3rd/4th	100
	1st instar larva	15	solenopsis mealybug	2nd/3rd	20
	2nd instar larva	12	solenopsis mealybug	2nd/3rd	40
	3rd instar larva	9	solenopsis mealybug	2nd/3rd	60 - 100

Adult green lacewing - survival and fecundity

First experiment

Newly emerged adults were placed into cylindrical plastic containers as male and female pairs (14.5 cm length x 9 cm diameter), with nylon gauze (14 cm square) attached to one end to provide ventilation. Diet, consisting of a mix of equal parts water, honey and yeast autolysate (Bugs for Bugs fruit fly lure) was provided three times a week as a small droplet added to the gauze with a paint brush. Each day survival and egg lay was recorded. Survival and fecundity data was collected on 9 pairs of lacewings.

Second experiment

In this experiment the diet was changed to include bee pollen which was diluted in water (1g: 9mL water), honey (1mL: 1mL water) and yeast autolysate, with each provided as separate droplets of approximately equal volume. Lacewings were initially setup as pairs, at 7 days old they were grouped together for mating, and then returned to containers as male: female pairs. Survival and fecundity data was collected for 14 pairs.

4.2 Prey choice studies

Three prey choice studies were completed with minute two-spotted ladybird. For both transverse ladybird and green lacewing a single study was completed (Table 3). The first two studies for minute two-spotted ladybird were designed to identify the most suitable developmental stage of whitefly to offer the predator in the choice experiment with cotton aphid.

Table 3. Summary of prey choice studies completed with each predator.

Experiment	Replicates	Predator		Prey	
		Species	Developmental stage	Species	Development stage
1	5	Minute two-spotted ladybird	adult	whitefly	eggs
					2nd instar
					adults
2	10	Minute two-spotted ladybird	adult	whitefly	1st instar
					4th instar
3	10	Minute two-spotted ladybird	adult	Whitefly	eggs
				cotton aphid	1^{st} – 2^{nd} instar
4	10	Transverse ladybird	adult	mealybug	2nd instar
				cotton aphid	2nd instar
5	10	Green lacewing	3rd instar larva	whitefly	3rd instar
				cotton aphid	3rd instar

Minute two-spotted ladybird

Experiment 1: Adult beetles were individually (n=5) released into an experimental arena (Petri dish 8 cm diameter) containing a cotton leaf with equal numbers (n= 20) of whitefly eggs, 2^{nd} instar nymphs and adults. Predation by the beetles was observed for 30 min and then beetles were left in the arenas for 24 h. The total number of prey consumed after 24h was recorded.

Experiment 2: Prey choice of 10 beetles was assessed by placing them individually in experimental arenas with 10 1^{st} instar and 10 4^{th} instar whitefly nymphs. The number of prey eaten was recorded after 24 h.

Experiment 3: Adult *D. notescens* prey choice in response to the presence of *B. tabaci* and *A. gossypii* was studied by placing them into an experimental arena (Petri dish 8 cm diameter) with 80 prey in the following ratios of whitefly to cotton aphid; 0:80, 20:60, 40:40, 60:20 and 80:0. Whiteflies were offered as eggs, based on earlier tests of life stage preference, and cotton aphids as 1^{st} to 2^{nd} instar nymphs. Treatment leaves were set up by confining female whiteflies with a clip cage to a cotton leaf for several hours to lay eggs. Location and number of eggs were marked and any excess eggs were removed. Cotton aphids were added to the leaf with a fine brush and left overnight to settle. Prior to adding the predator, prey numbers were checked and additional aphids added if needed. For each treatment (prey ratio) 10 replicates were conducted, with one adult ladybeetle per replicate. After five hours the number of remaining cotton aphids and whitefly eggs were counted for each replicate. For each treatment, two controls were set up where a ladybeetle was not added so mortality could be corrected based on numbers left in the control after 5 hours.

Transverse ladybird

Experiment 4: The prey choice of adult transverse ladybeetles was studied in a laboratory experiment by offering beetles different densities of second instar cotton aphids and solenopsis mealybug, *Phenacoccus solenopsis* Tinsley. Five treatments, each a different ratio

of prey types; 0:20, 5:15, 10:10, 15:5 and 20:0 mealybug to aphid were offered to the ladybeetles for a period of three hours. The number of prey consumed was recorded at the end of the exposure period. Each treatment was replicated 10 times which each replicate comprising an individual ladybeetle. Each ladybeetle was offered each treatment over a period of two days, and the order treatments were offered to each ladybeetle was randomised. Prior to testing, the ladybeetles were provided water and honey but otherwise starved of prey for approximately 15 hours. Overnight between the first and second day of the experiment the ladybeetles were kept in Petri dishes with water, but no prey.

Green Lacewing

Experiment 5: Prey selection by *M. signatus* when offered both *A. gossypii* and *B. tabaci* nymphs at different densities was recorded in a laboratory experiment. Five prey density treatments were offered to *M. signatus* 3rd instar larvae for 10 hours and each treatment was replicated 10 times. The treatments were prey ratios of *B. tabaci* to *A. gossypii* 3rd instar nymphs with the total prey density remaining the same. The treatments consisted of 100 prey items at the following ratios of *B. tabaci* to *A. gossypii*; 0:100, 25:75, 50:50, 75:25 and 100:0.

The lacewing larvae were purchased from a commercial insectary (Bugs for Bugs) as eggs and to prevent prior experience with either prey tested in the experiment, they were reared on *P. solenopsis.* Before the start of the experiment, the lacewing larvae were deprived of prey for around 10 hours.

The lacewing larvae were transferred into the Petri dish experimental arenas with a fine paint brush and allowed 10 hours to consume prey, before being removed and the number of remaining prey recorded.

Data analysis

2.2 Test whitefly for resistance

Bioassays were analysed using probit analysis with data corrected for control mortality (Abbots formula). Genstat 16.1 was used to complete all statistical tests. Assays with high control mortality (> 5%) or high replicate variability were repeated.

3.1 Test toxicity of insecticides on whitefly parasitoid

Data was corrected for control mortality (Schneider-Orelli formula) then arcsine transformed, before being analysed in a 2 way ANOVA with insecticide and exposure time as factors. Means were separated by least significant difference test after a significant F test at $p = 0.05$.

4.1 Prey consumption studies

For minute two-spotted ladybird adult aphid consumption data was corrected for control mortality using the Henderson-Tilton formula and then a one way analysis of variance (ANOVA) was completed to test for differences in female and male daily aphid consumption.

For minute two-spotted ladybird and green lacewing age-specific survival (l_x) and fertility (m_x) were calculated.

4.2 Prey Choice Studies

Predator preference was calculated by Manly's preference index.

$$\beta_1 = \frac{\ln\left(\frac{e_1}{A_1}\right)}{\ln\left(\frac{e_1}{A_1}\right) + \ln\left(\frac{e_2}{A_2}\right)}$$

Where the index β_1 is the predators' preference for prey type 1, e_1 is the number of surviving prey belonging to prey type 1, A_1 is the number of prey type 1 offered, e_2 is the number of prey type 2 remaining and A_2 is the number of prey type 2 offered. The value of β_1 will fall between 0 and 1. An index close to 1 indicates preference for prey type 1 and an index close to 0 indicates preference for prey type 2. A value close to 0.5 indicates the predator selects prey randomly, showing no preference.

The preference of minute two-spotted ladybird for one type of prey over the other was tested by comparing the β_1 values of using an ANOVA followed by least significant difference test. Prey switching in *D. notescens* was tested using a Student's t-test that compered the estimated β_1 values with expected values.

All analysis was completed in Genstat 16.1.

Results

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

1. Identification of whitefly species/biotype

Subsamples of whitefly collected from cotton growing regions of QLD and NSW were identified using molecular methods (Table 4). In total 123 *B. tabaci* individuals were sequenced and all were shown to be a single haplotype of *B. tabaci* MEAM1 (B biotype). All were genetically identical (100% similarity) over the 657 bp 3' barcode COI sequence. One representative sequence of this MEAM1 haplotype was selected and used for the phylogenetic inference study.

- For the 2014 season, 18 adults were sequenced from 14 locations and all were *B. tabaci* Middle East Asia Minor 1 species (*B. tabaci* MEAM1).
- For the 2015 season, 39 adults were sequenced from 15 locations and all were *B. tabaci* MEAM1.
- For the 2016 season, 66 adults were sequenced from 17 locations and all were *B. tabaci* MEAM1.

A pairwise comparison of COI sequences of the global *B. tabaci* MEAM1 genotypes represented in the MEAM1 clade of revealed a sequence similarity of 97.11-100% with reference to the Australian cotton *B. tabaci* MEAM1 haplotype sequence, with a divergence of 0-3.81% among the sequences.

Phylogenetic inference

A phylogenetic reconstruction of the genetic relatedness of various *B. tabaci* genotypes from around the world is presented (Figure 1). The COI sequences from the 123 adult samples from this study were represented as a single haplotype sequence named "Australia MEAM1 Haplotype_Cotton_2014-16", and this sequence grouped within the monophyletic *B. tabaci* Middle East Asia Minor 1 species grouping.

Table 4. Collection details for whitefly samples (2014-2016)

Site Code	Host Plant	Locality	State	Date of Collection	Collector
EM14-1	Cotton	Emerald	QLD	21/01/2014	J. Hopkinson
EM14-2	Cotton	Emerald	QLD	21/01/2014	J. Hopkinson
MA14-1	Cotton	Toobeah	QLD	19/03/2014	J. Hopkinson
MA14-2	Cotton	Boggabilla	NSW	19/03/2014	J. Hopkinson
MA14-3	Cotton	Boggabilla	NSW	19/03/2014	J. Hopkinson
SG14-1	Cotton	St George	QLD	14/02/2014	J. Hopkinson
SG14-2	Cotton	St George	QLD	14/02/2014	J. Hopkinson
SG14-3	Cotton	St George	QLD	14/02/2014	J. Hopkinson
TH14-1	Cotton	Theodore	QLD	21/01/2014	J. Hopkinson
NM14-1	Cotton	Wee Waa	NSW	12/03/2014	S. van Brunschot
NM14-1	Cotton	Wee Waa	NSW	12/03/2014	S. van Brunschot
NM14-2	Cotton	Wee Waa	NSW	12/03/2014	S. van Brunschot
MO14-1	Cotton	Moree	NSW	12/03/2014	S. van Brunschot
MO14-2	Cotton	Moree	NSW	12/03/2014	S. van Brunschot
EM15-1	Cotton	Emerald	QLD	21/01/2015	P. Grundy
EM15-2	Cotton	Emerald	QLD	21/01/2015	P. Grundy
TH15-1	Cotton	Theodore	QLD	03/03/2015	J. Hopkinson
SG15-1	Cotton	St George	QLD	24/02/2015	J. Hopkinson
SG15-2	Cotton	St George	QLD	24/02/2015	J. Hopkinson
SG15-3	Cotton	St George	QLD	24/02/2015	J. Hopkinson
GR15-1	Melon	Griffith	NSW	26/03/2015	Jianhua Mo
NM15-1	Cotton	Wee Waa	NSW	31/03/2015	J. Hopkinson
NM15-2	Cotton	Narrabri	NSW	01/04/2015	J. Hopkinson
MO15-1	Cotton	Moree	NSW	01/04/2015	J. Hopkinson
MO15-2	Cotton	Moree	NSW	01/04/2015	J. Hopkinson
DD15-1	Cotton	Cecil Plains	QLD	09/04/2015	P. Grundy

Site Code	Host Plant	Locality	State	Date of Collection	Collector
MA15-1	Cotton	Boggabilla	NSW	09/04/2015	J. Hopkinson
MA15-2	Cotton	Boggabilla	NSW	09/04/2015	J. Hopkinson
MA15-2	Cotton	Boggabilla	NSW	09/04/2015	J. Hopkinson
EM16-1	Cotton	Emerald	QLD	25/02/2016	P. Grundy
TH16-1	Cotton	Theodore	QLD	25/02/2016	P. Grundy
TH16-2	Cotton	Theodore	QLD	25/02/2016	P. Grundy
SG16-1	Cotton	St George	QLD	09/02/2016	J. Hopkinson
SG16-2	Cotton	St George	QLD	09/02/2016	J. Hopkinson
SG16-3	Cotton	St George	QLD	09/02/2016	J. Hopkinson
MU16-1	Cotton	Mungindi	NSW	14/03/2016	J. Hopkinson
NM16-1	Cotton	Wee Waa	NSW	09/03/2016	J. Hopkinson
NM16-2	Cotton	Wee Waa	NSW	09/03/2016	J. Hopkinson
MO16-1	Cotton	Moree	NSW	11/02/2016	P. Grundy
MO16-2	Cotton	Moree	NSW	11/02/2016	P. Grundy
Croppa Creek	Cotton	Croppa Creek	NSW	29/03/2016	M. Stone
MA16-1	Cotton	Boggabilla	NSW	03/02/2016	J. Hopkinson
MA16-2	Cotton	Boggabilla	NSW	03/02/2016	J. Hopkinson
MA16-3	Cotton	Boggabilla	NSW	03/02/2016	J. Hopkinson
MA16-4	Cotton	Boomi	NSW	14/3/2016	J. Hopkinson
HI16-1	Cotton	Hillston	NSW	03/03/2016	E. Storrier

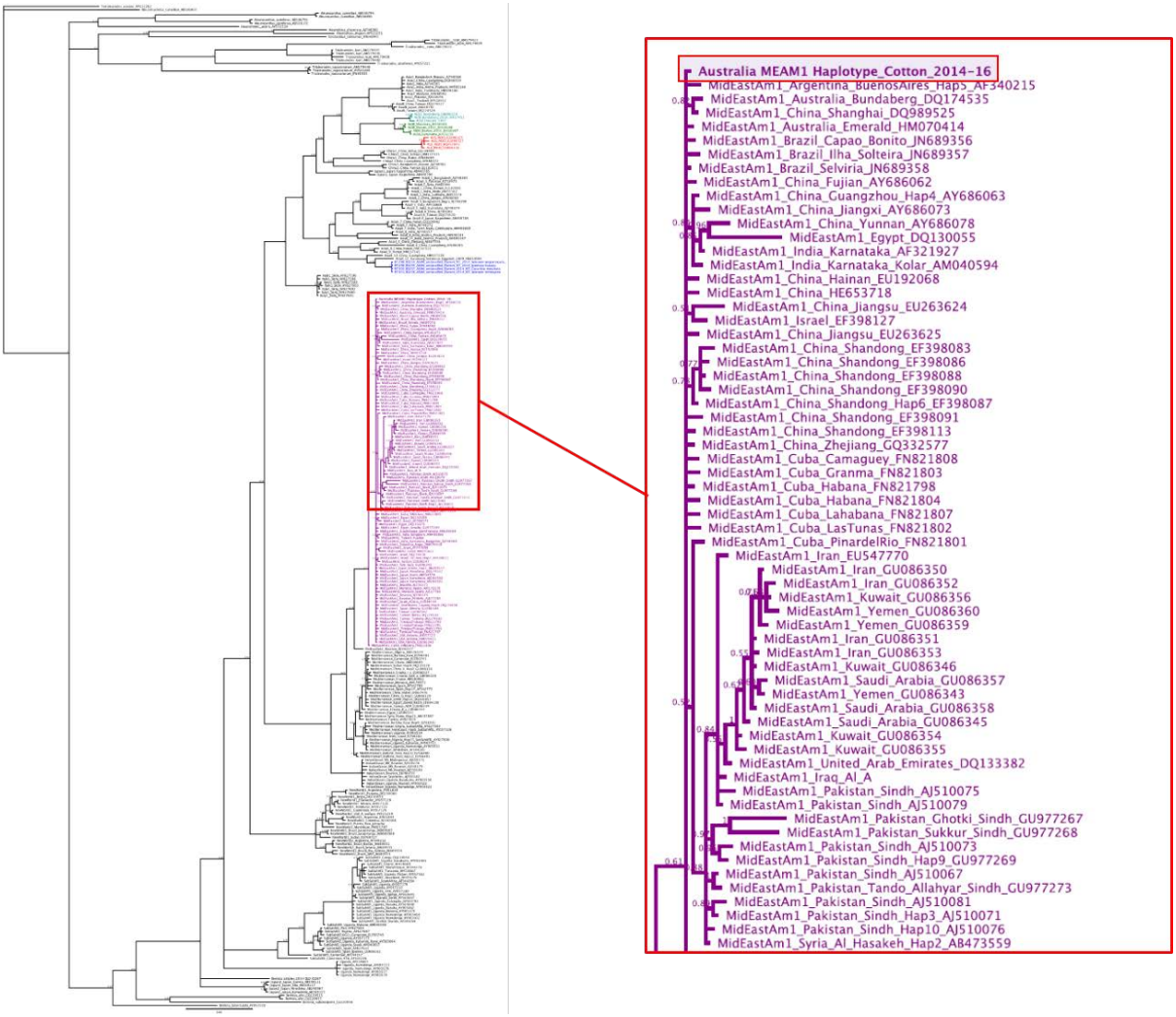

Figure 1. Bayesian phylogeny of global representative species of the *Bemisia tabaci* species complex. Highlighted in red is the positioning of the *Bemisia tabaci* collected in cotton (Australia MEAM1 Haplotype_Cotton_2014-16) in the Middle East Asia Minor 1 species grouping.

2. Insecticide resistance of silverleaf whitefly

Resistance levels to each registered insecticide were tested for each season. Additional testing was completed for sulfoxaflor and clothianidin. The following codes have been used to identify localities; SUS = Susceptible lab strain, EM = Emerald, TH = Theodore, SG = St George, MU = Mungindi, DD = Darling Downs, MA = Macintyre Valley, MO = Moree, NM = Namoi Valley, GR = Griffith and HI = Hillston.

Pyriproxyfen (Admiral®)

Bioassay testing of pyriproxyfen found that one strain from Croppa Creek in 2016 was resistant with a LC_{50} resistance factor of 67 when tested in the F2 generation (Figure 2, Figure 3 & Appendix 1). When tested again at the F4 generation the Croppa Creek strain (Croppa Creek 2 in figures) was still resistant with an LC_{50} resistance factor of 27. Pressuring in the glasshouse at 20 ppm (double tolerance endpoint) and then at 100 ppm resulted in survivors, confirming resistance individuals were present in the Croppa Creek strain. High

tolerance was found in three strains; MU16-1 (Mungindi), MA16-2 (Boggabilla) in 2016 and NM15-2 (Narrabri) in 2015. Pressuring of MA16-2 at 20 ppm did not result in survivors, which indicates this strain was not resistant. All other strains tested were susceptible.

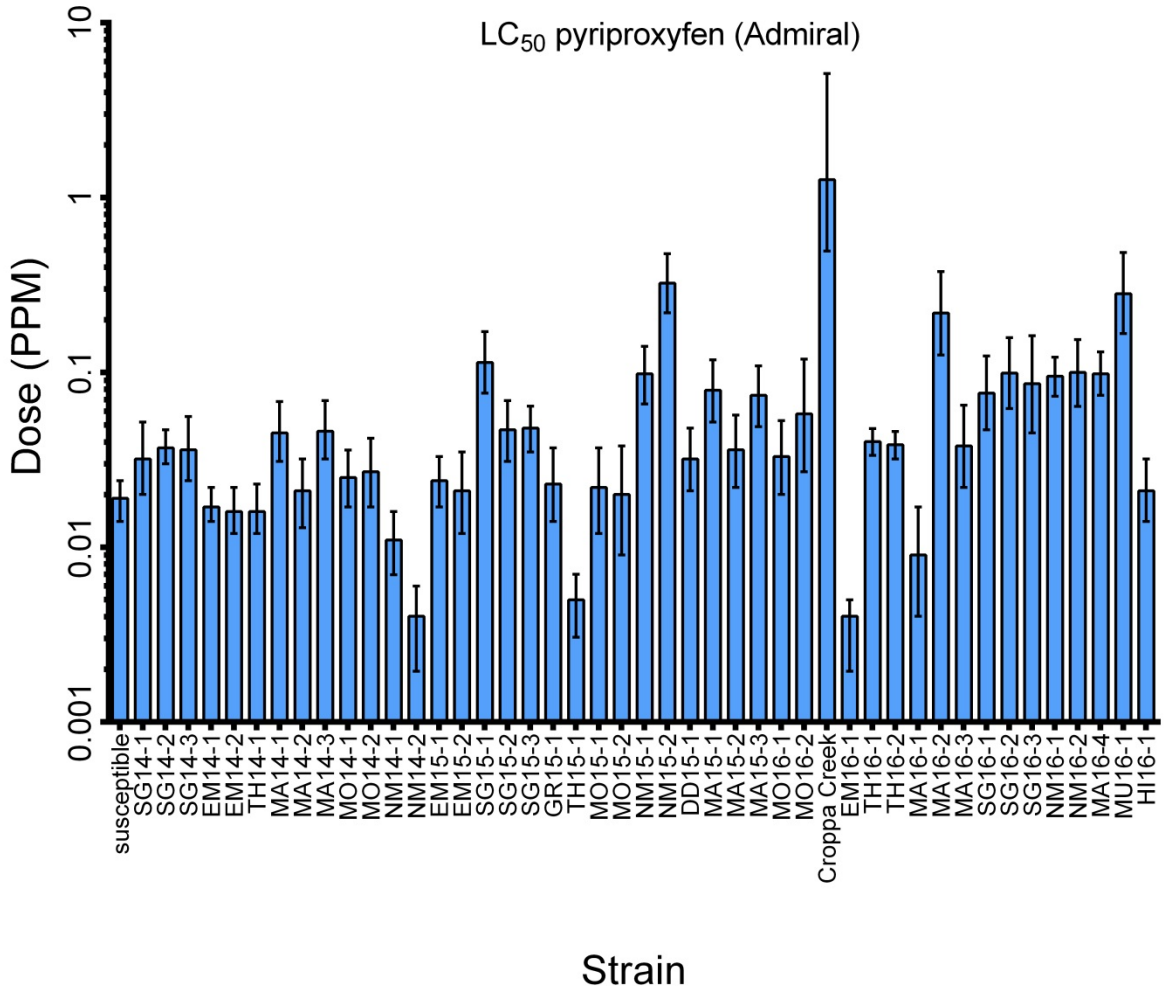

Figure 2. Dose of pyriproxyfen required to reach LC_{50} for each SLW strain tested. Reference lab susceptible strain is shown in the first 2 columns.

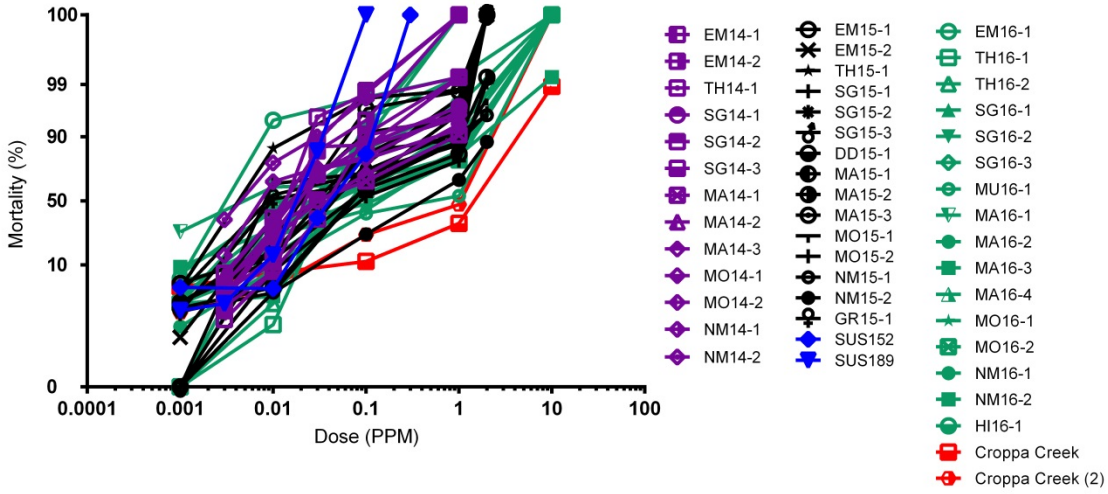

Figure 3. Dose response mortality of each SLW strain tested to pyriproxyfen.

Diafenthiuron (Pegasus®)

Testing of diafenthiuron showed all SLW strains were susceptible with LC_{50} and $_{90}$ values close to the lab susceptible (Figure 4 & Appendix 2). No changes in susceptibility of SLW to diafenthiuron have been detected over the course of the project (Figure 5) and the results are comparable to those in the previous project.

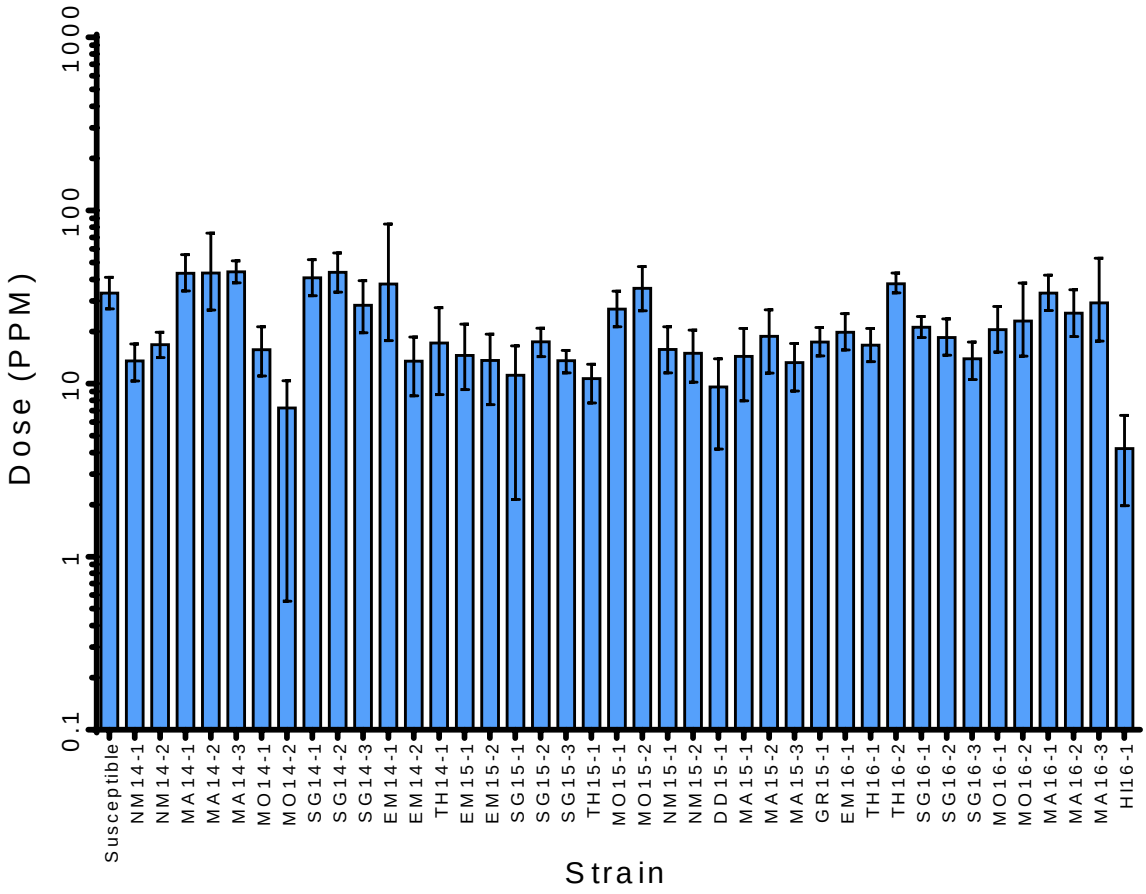

Figure 4. Dose of diafenthiuron required to reach LC_{50} for each SLW strain tested. Reference lab susceptible strain is shown in the first column.

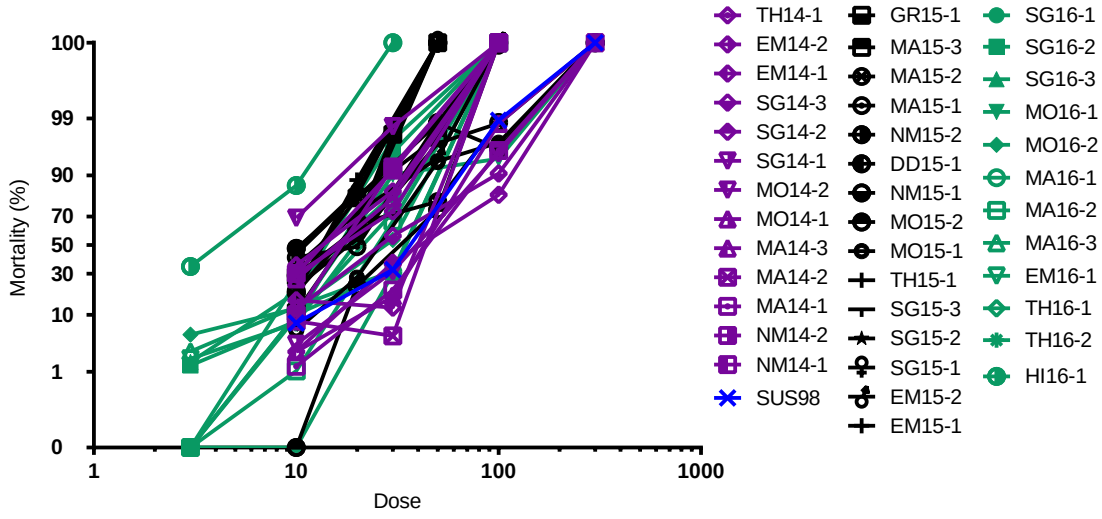

Figure 5. Dose response mortality of each SLW strain tested to diafenthiuron.

Spirotetramat (Movento®)

Bioassay results for spirotetramat indicate all strains tested are susceptible with no difference detected between the susceptible lab strain and field strains (Figure 6 & Appendix 3). The original dosage range did not satisfactorily cover field strain tolerance during testing in 2014; as a result the upper dose tested has been increased from 30 ppm in 2014 to 100 in 2015 to 300 ppm in 2016 (Figure 7).

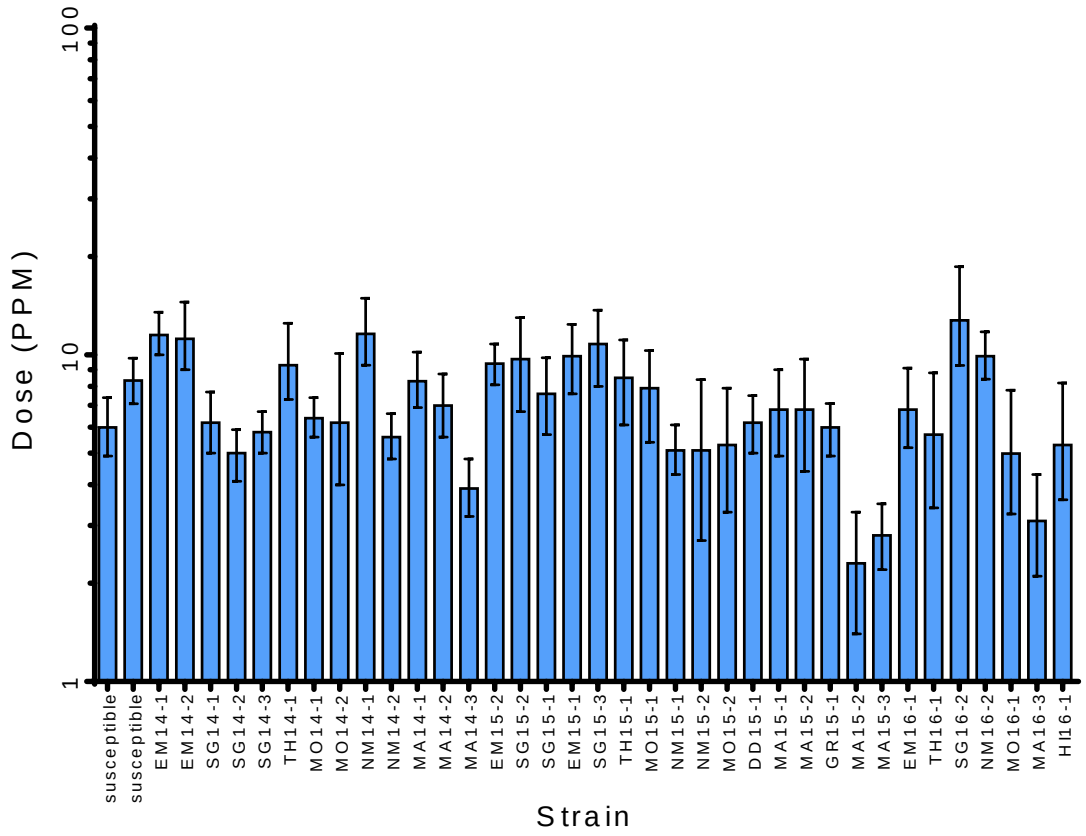

Figure 6. Dose of spirotetramat required to reach LC_{50} for each SLW strain tested. Reference lab susceptible strain is shown in the first 2 columns on the left.

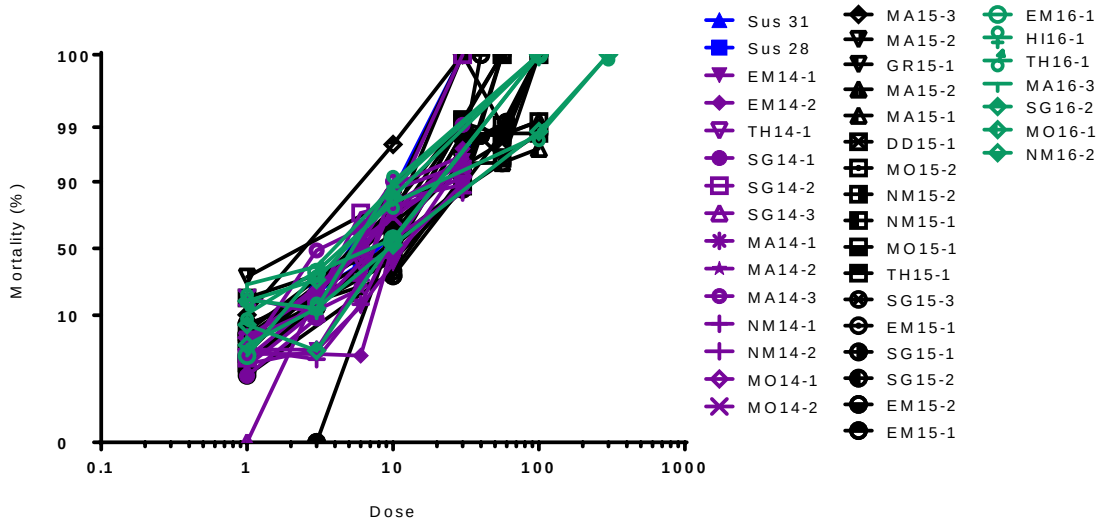

Figure 7. Dose response mortality of each SLW strain tested to spirotetramat.

Cyantraniliprole (Exirel®)

All strains tested in cyantraniliprole bioassays were susceptible (Figures 8, 9 & Appendix 4). An original discriminating dose of 0.1 ppm was recommended at the conclusion of the baseline cyantraniliprole study. Our subsequent testing would indicate this is too low and a safer level would be 1 ppm. Testing particularly in 2015 and 2016 show susceptible populations often need a dose above 0.1 to be 100% killed. Differences between the original baseline study and current resistance testing are likely due to differences in assessment and due to new methodology adopted for the 2015/16 testing.

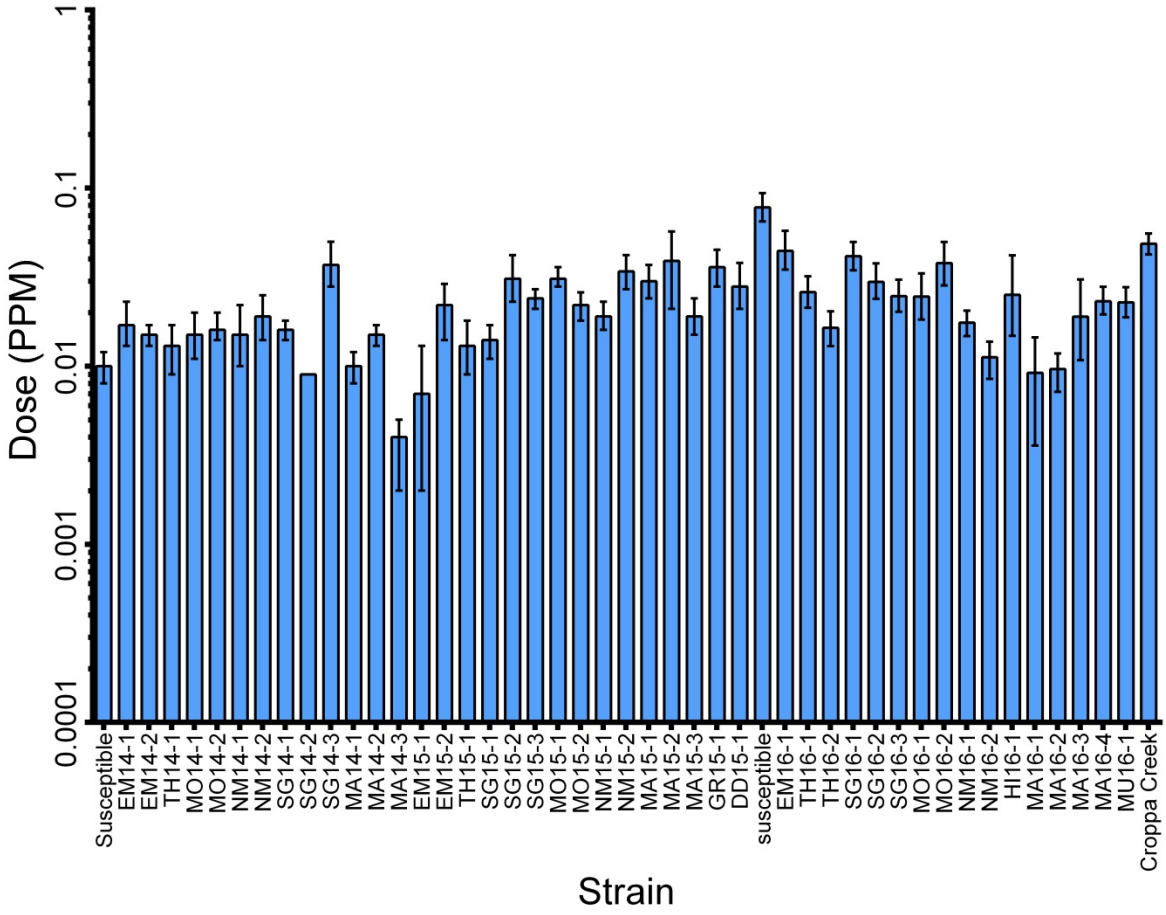

Figure 8. Dose of cyantraniliprole required to reach LC_{50} for each SLW strain tested. Reference lab susceptible strain is shown in the first column.

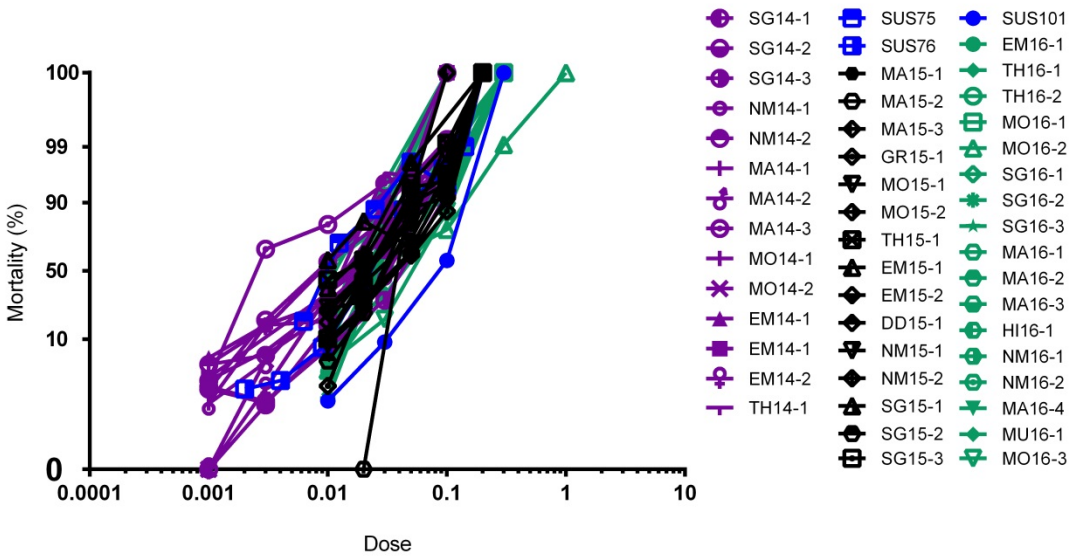

Figure 9. Dose response mortality of each SLW strain tested to cyantraniliprole.

Dinotefuran (Starkle®)

Dinotefuran is a newly registered insecticide for the control of mirids and SLW in cotton. During the 2014/15 season dinotefuran was tested against a susceptible lab strain, resistant lab strains (bifenthrin and pyriproxyfen) and field strains. Two bioassay types were used; a systemic uptake method (Figure 10 & Appendix 5) and a foliar leaf dip method (Figure 11 & Appendix 6). While the systemic method is more sensitive than the foliar method, the foliar method is more representative of field application. For the 2015/16 season the foliar method was used alone (Figure 12). Baseline data from the two seasons of testing would indicate the discriminating dose should be set at 320 ppm, application rates are 250 – 375 g/ha (500 – 750 ppm) for SLW and 90 g/ha (180 ppm) for mirids. In theory widespread use of the low rate for mirids has the potential to select for resistance in SLW. If SLW are present when spraying for mirids it is recommended to use the SLW rate.

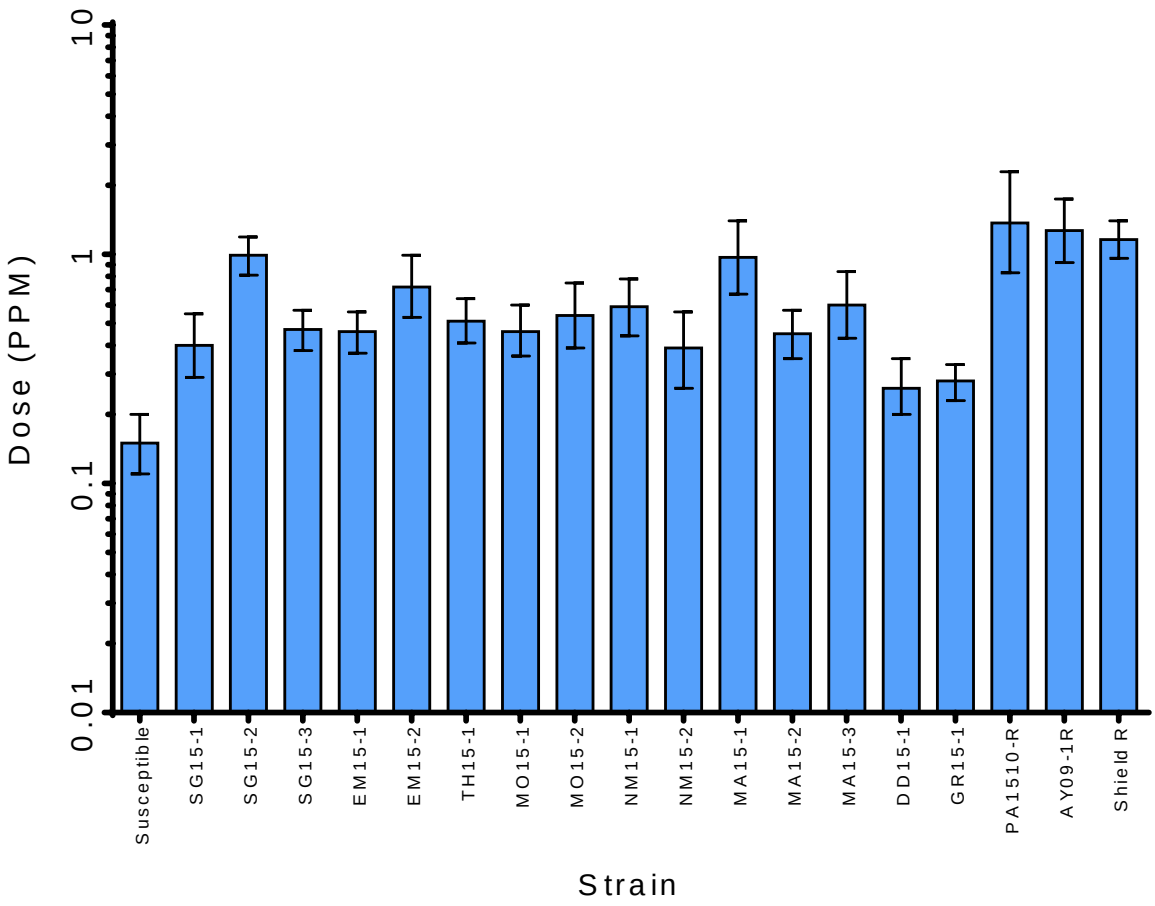

Figure 10. Dose of dinotefuran in a systemic assay required to reach LC_{50} for each SLW strain tested. Reference lab susceptible strain is shown in the first column.

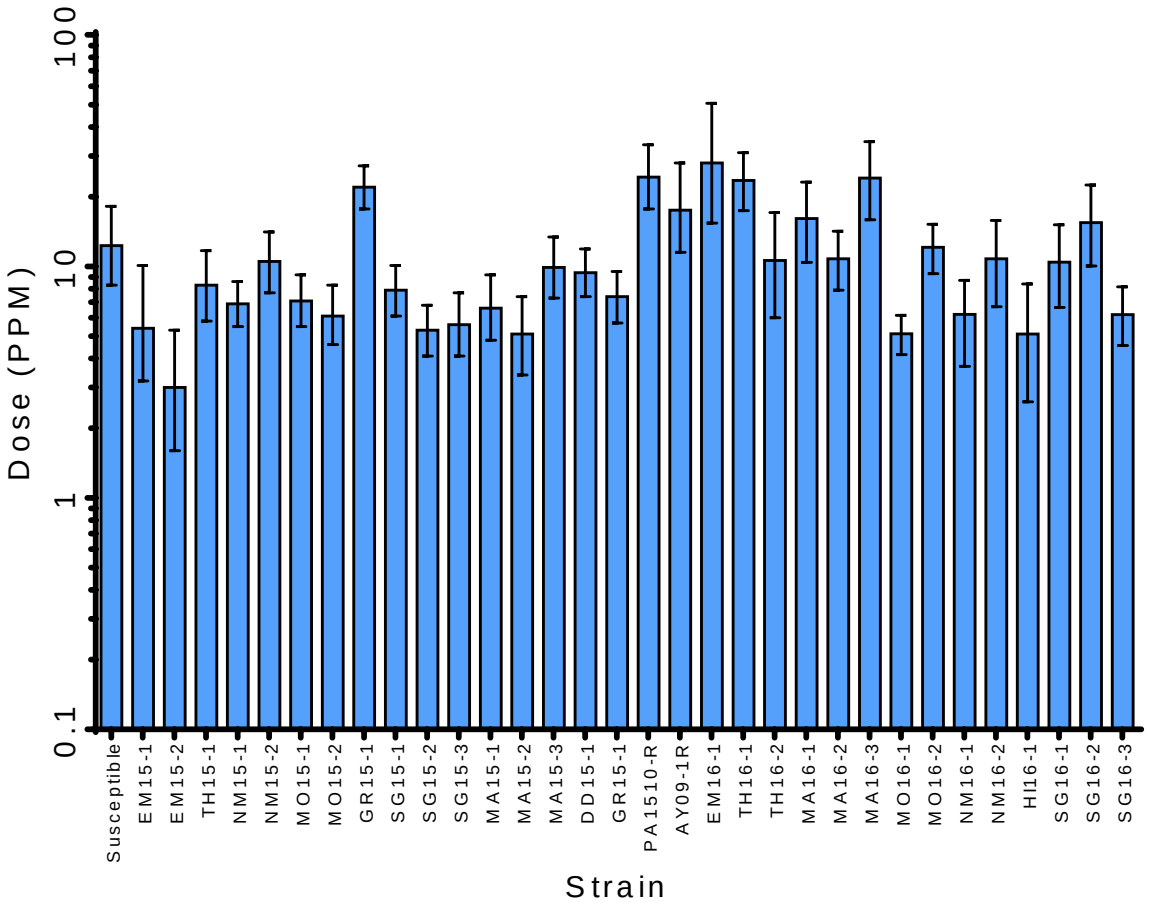

Figure 11. Dose of dinotefuran in a foliar assay required to reach LC_{50} for each SLW strain tested. Reference lab susceptible strain is shown in the first column.

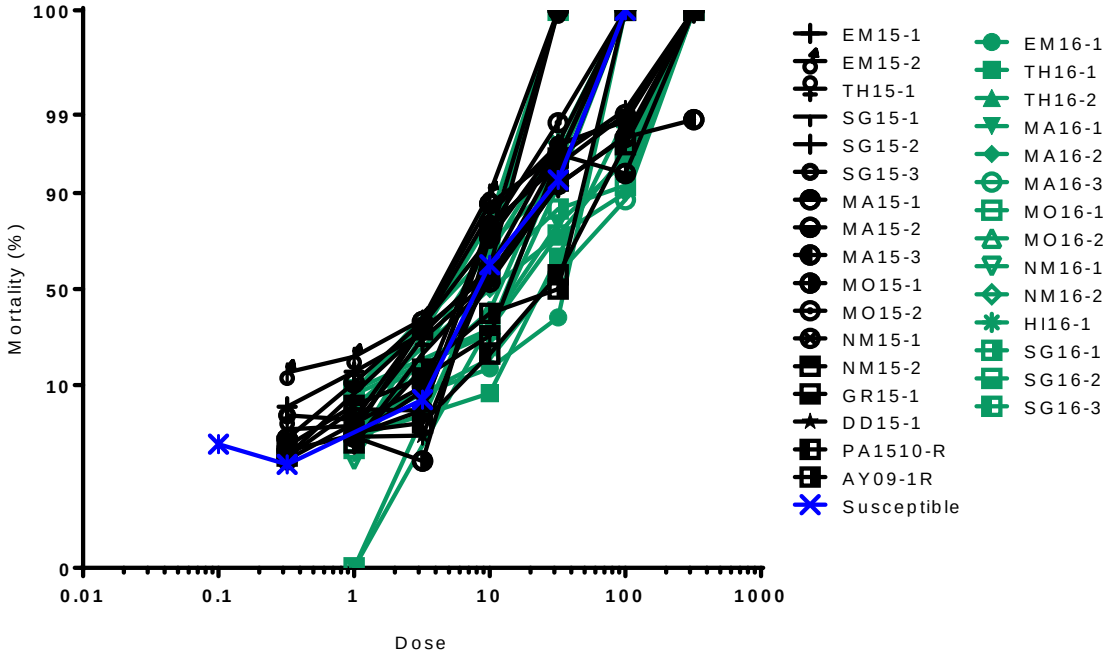

Figure 12. Dose response mortality of SLW in foliar dinotefuran bioassays.

Bifenthrin (Talstar®)

Bioassays testing of bifenthrin did not show any evidence of resistance in populations tested (Figure 13). Overall the results are similar to those in the previous 3 years (2010 – 13). At LC_{50} the highest resistance factor detected was a strain from Moree (MO14-2) in 2013/14 with a value of 6.3 (Appendix 7). In 2015/16 a strain from Macintyre (MA16-1) showed signs of potential resistance with a LC_{50} resistance factor of 5.5. This strain was pressured with a dose of 1000 ppm but no adults survived this dose, indicating it was susceptible. In 2015/16 the dose required to control 100% of individuals tested for strains was generally either 100 or 320 ppm, but in two cases (MA16-1 & HI16-1) a dose of 1000 ppm was required (Figure 14) which is above the field rate of 800 ppm.

In 2015 a change was made to the insecticide resistance management strategy to reduce the usage of bifenthrin from 2 sprays per season to 1.

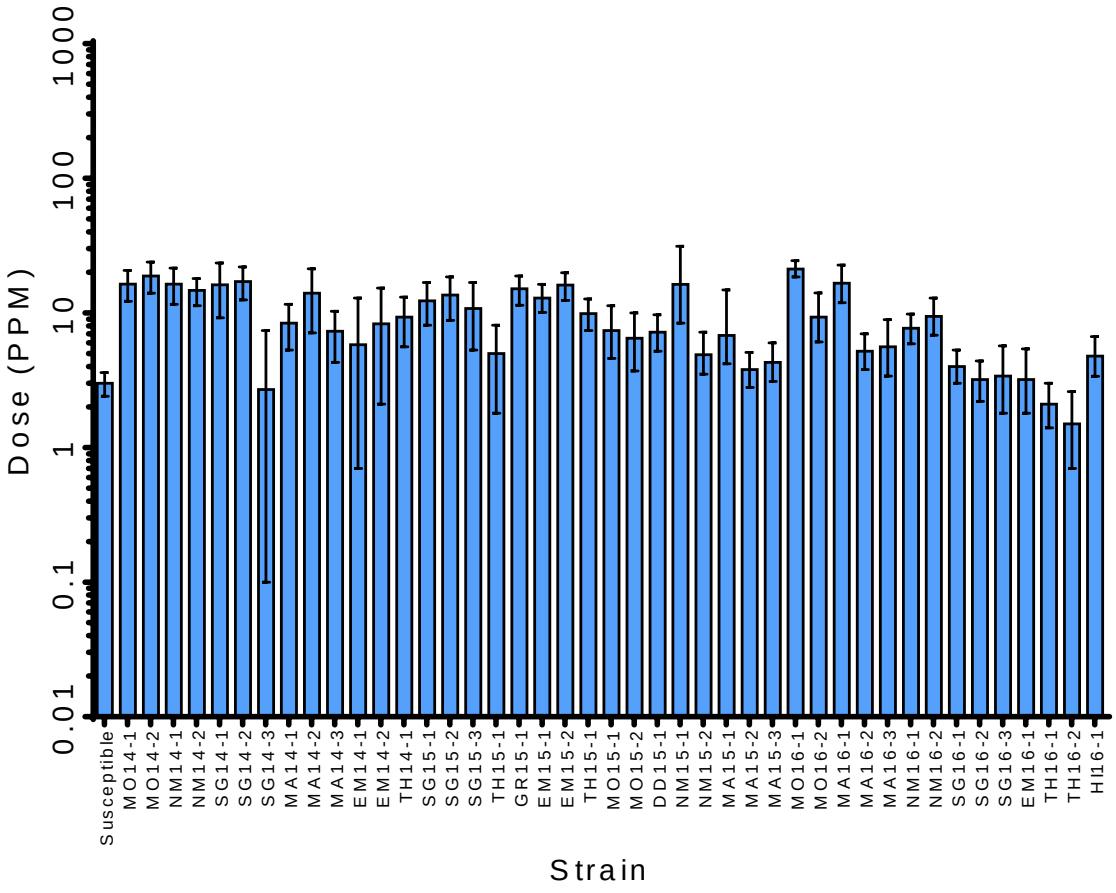

Figure 13. Dose of bifenthrin required to reach LC_{50} for each SLW strain tested. Reference lab susceptible strain is shown in the first column.

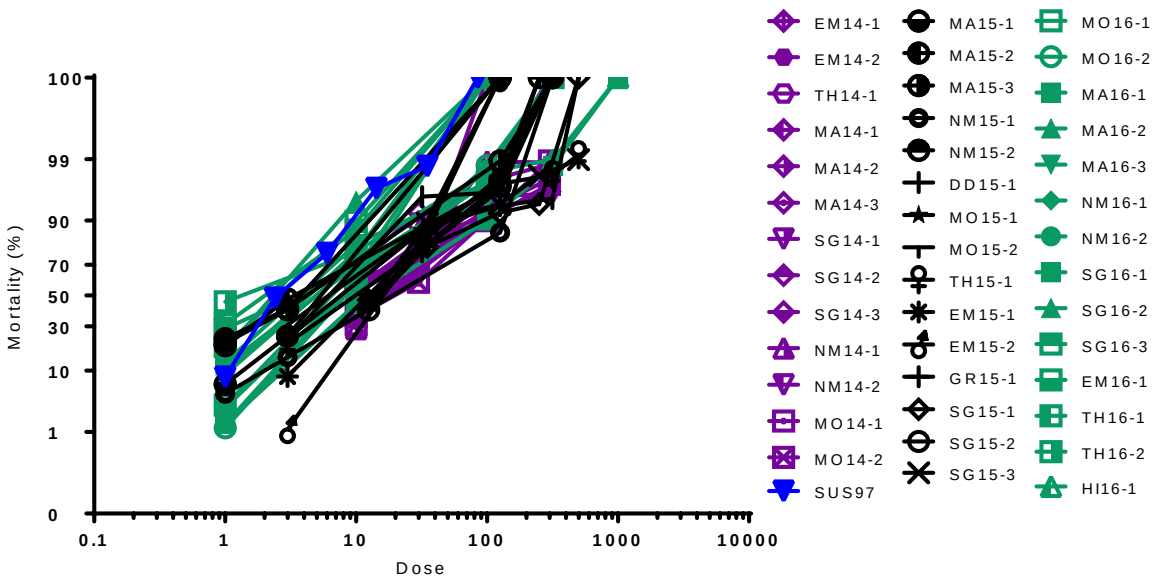

Figure 14. Dose response mortality of each SLW strain tested to bifenthrin.

Clothianidin (Shield®)

Clothianidin is not registered for SLW control, but is a commonly used insecticide in cotton and as such SLW is widely exposed to this insecticide. For foliar assays the resistance factor at LC_{50} shows only two populations with resistance, SG15-3 (St George) and GR15-1 (Griffith) (Figure 15). However as the dose response to clothianidin is flat for most field strains collected in 2015 and 2016, the LC_{50} value is not the best level to compare strains (Figure 16). If the resistance factor at LC_{90} is used it shows that most populations have resistance (Figures 17 & Appendix 8).

Contrary to the foliar assay results, systemic assays that tested field strains from 2015 showed no evidence of resistance (Appendix 9). A glasshouse study in 2016 confirmed the foliar method was comparable to a boom spray application. It appears the foliar leaf dip method does not allow enough insecticide contact with the insect, but as this method most reflects field application we have continued to use it.

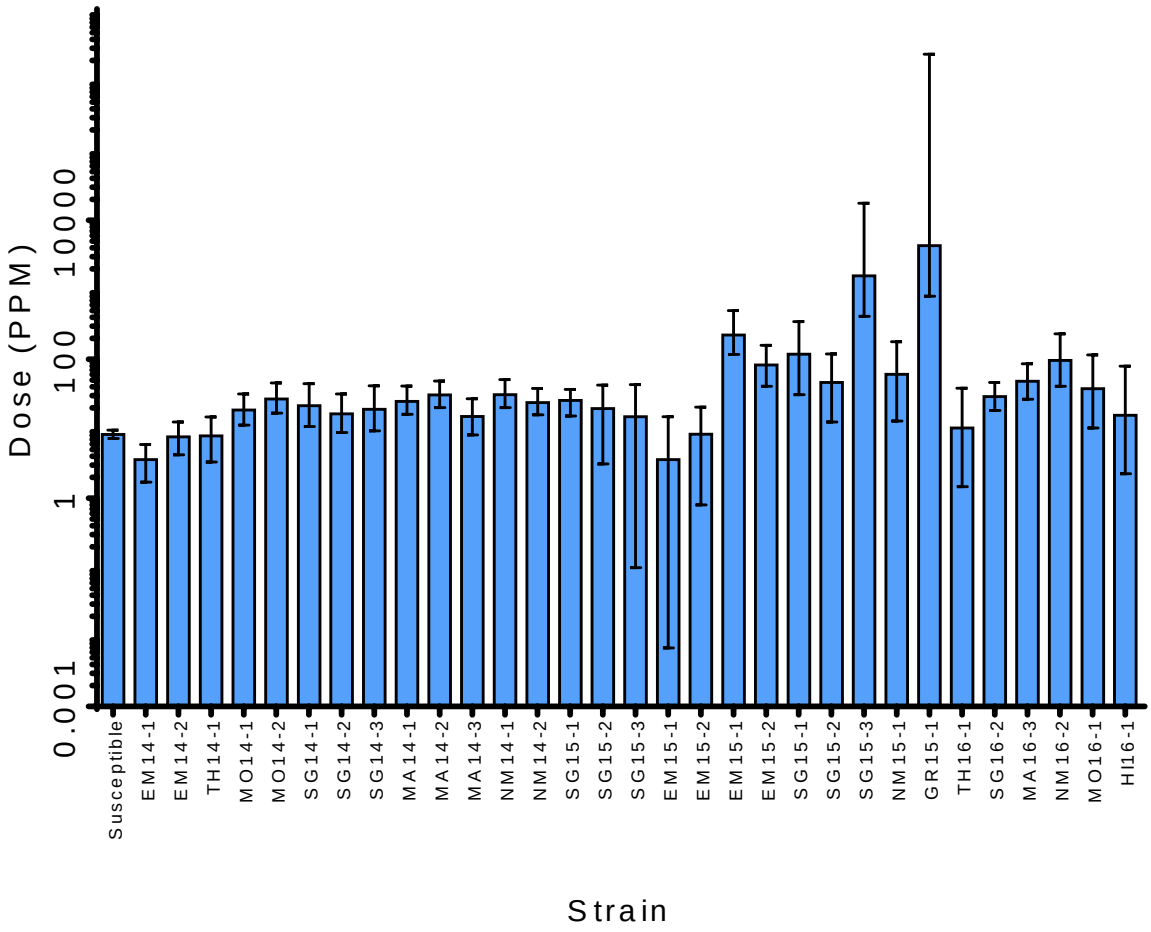

Figure 15. Dose of clothianidin required to reach LC_{50} for each SLW strain tested. Reference lab susceptible strain is shown in the first column.

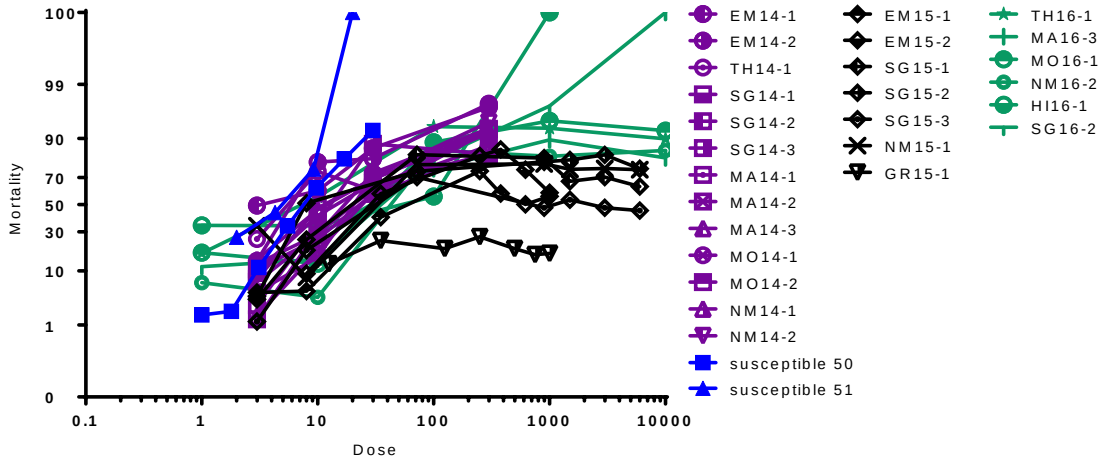

Figure 16. Dose response mortality of each SLW strain tested in foliar clothianidin assays.

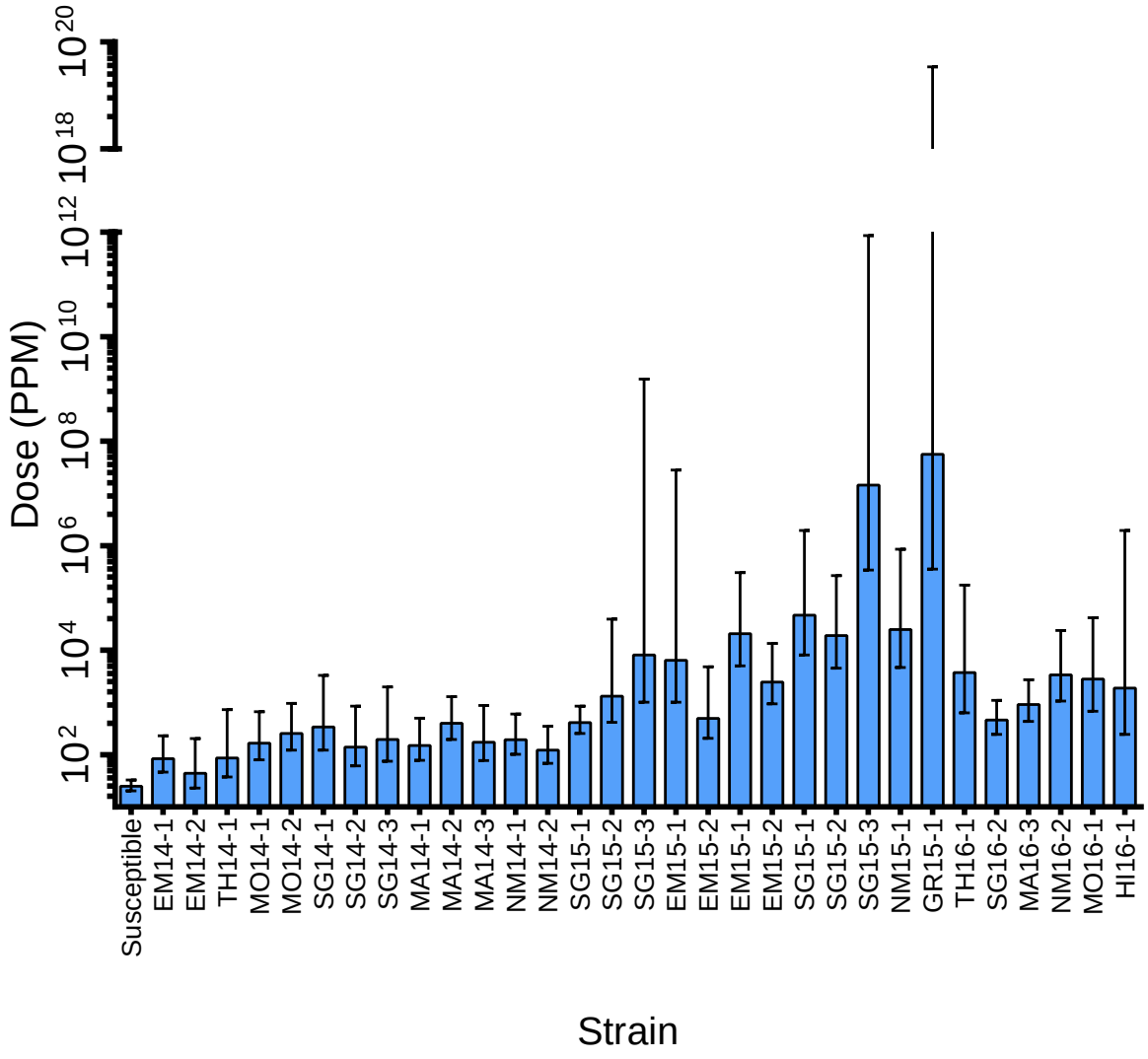

Figure 17. Dose of clothianidin required to reach LC_{90} for each SLW strain tested. Reference lab susceptible strain is shown in the first column.

Sulfoxaflor (Transform™)

Sulfoxaflor is a recently registered insecticide in cotton for aphid, green mirid and greenhouse whitefly control, but not SLW. All strains tested in 2013/14 and 2014/2015 were susceptible (Figures 18, 19 & Appendix 10).

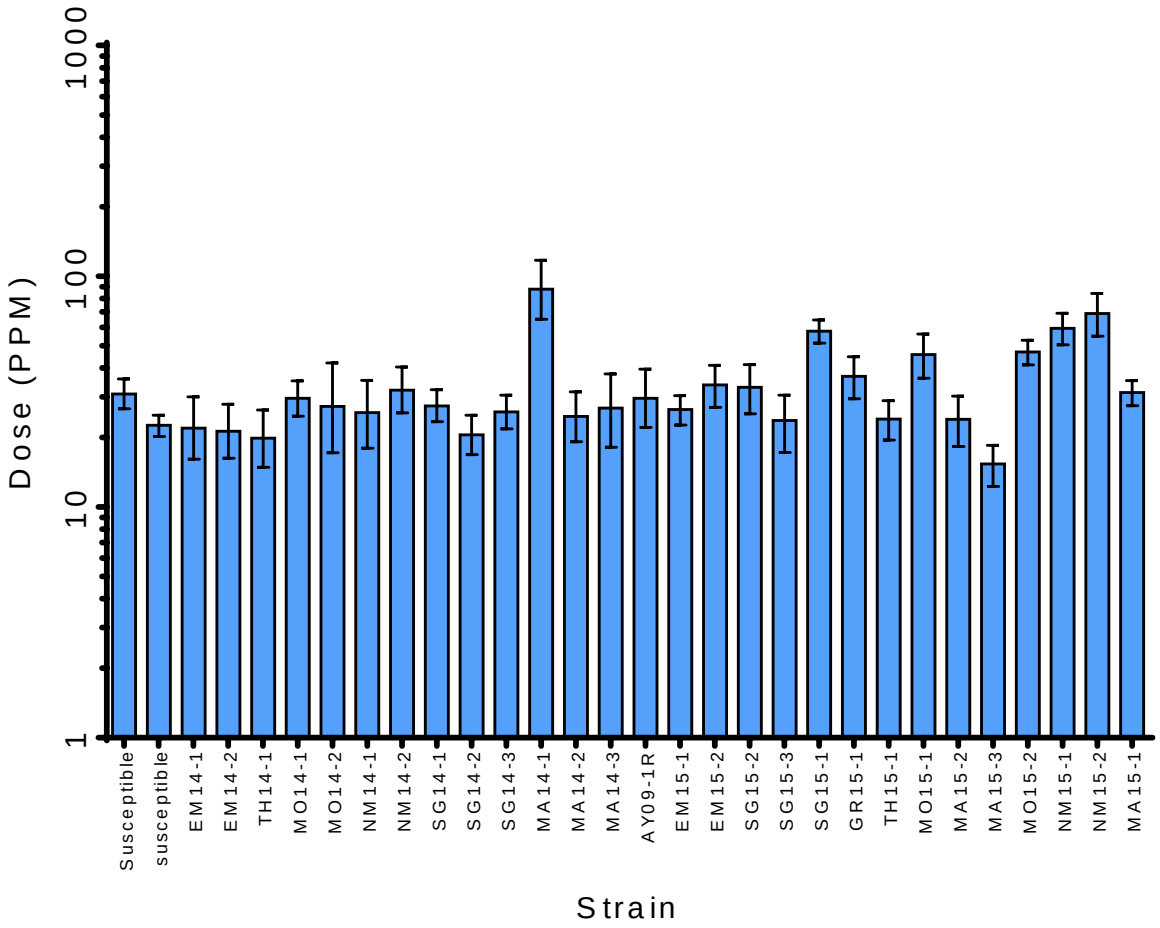

Figure 18. Dose of sulfoxaflor required to reach LC_{50} for each SLW strain tested. Reference lab susceptible strain is shown in the first 2 columns.

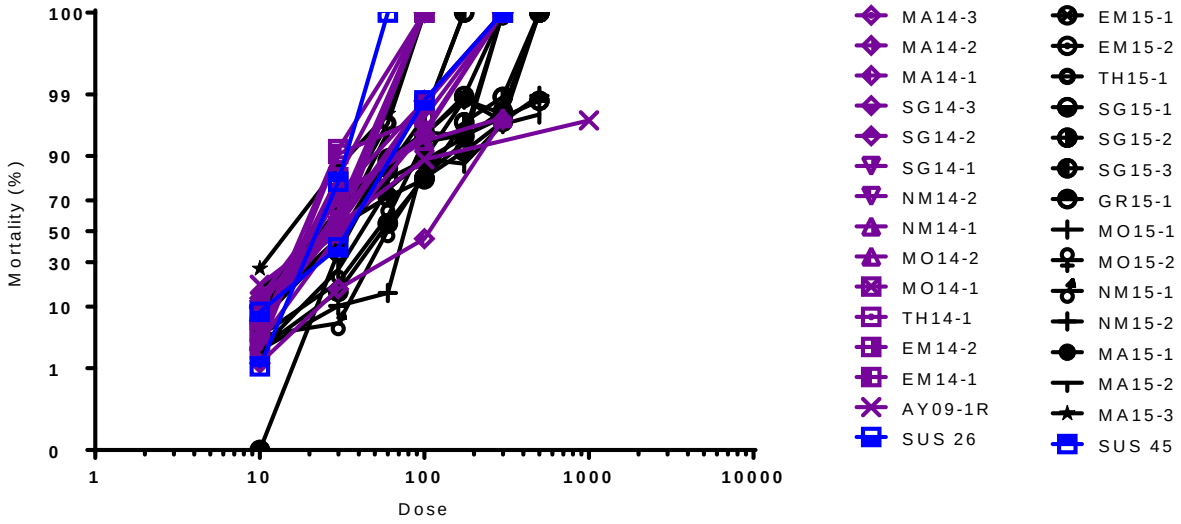

Figure 19. Dose response mortality of each SLW strain tested to sulfoxaflor.

3. Insecticide impacts on the whitefly parasitoid *Eretmocerus hayati*

Toxicity of insecticides was assessed after direct exposure and exposure to dried residue at 3 time intervals.

Direct exposure

Direct exposure to sulfoxaflor, dinotefuran, clothianidin and bifenthrin resulted in high mortality, compared to moderate mortality with fipronil, cyantraniliprole and spirotetramat. The flonicamid treatment had the lowest mortality but it wasn't significantly different to spirotetramat or cyantraniliprole (Table 5).

Exposure to residue

Bifenthrin, clothianidin and both rates of dinotefuran had very high toxicity. The neonicotinoids (clothianidin and dinotefuran) were persistent to 8 days, will the toxicity of bifenthrin dropped significantly between 2 and 8 days (Table 5). Both fipronil and sulfoxaflor were moderately toxic. The toxicity of sulfoxaflor declined significantly between 2 and 8 days, while fipronil was still moderately toxic at 8 days. Toxicity to cyantraniliprole was low to moderate with highest the residue toxicity observed on day 2. Flonicamid and spirotetramat both had low toxicity, killing less than 20% of wasps.

Table 5. *Eretmocerus hayati* mortality (%) ± SE after exposure to insecticides. Values sharing the same letters are not significantly different (P<0.05).

Insecticide	direct exposure	1d residue	2d residue	8d residue
Flonicamid	15.3 ± 4.0 ghij	13.2 ± 3.0 ghij	8.6 ± 7.1 ij	3.7 ± 2.5 ij
Spirotetramat	24.7 ± 6.4 fghi	10.6 ± 8.7 hij	19.1 ± 12.8 fghij	0.6 ± 3.9 j
Cyantraniliprole	23.8 ± 6.4 fghi	13.1 ± 12.0 ghij	32.5 ± 5.7 efg	10.5 ± 7.0 hij
Fipronil	37.8 ± 4.7 ef	21.6 ± 5.8 fghij	15.9 ± 7.8 ghij	30.3 ± 9.4 efgh
Sulfoxaflor	73.1 ± 5.9 bcd	23.2 ± 5.5 fghi	33.2 ± 7.2 efg	5.5 ± 3.8 ij
Dinotefuran (90)	74.6 ± 1.0 bcd	65.9 ± 6.1 d	69.9 ± 7.5 cd	78.9 ± 3.4 bcd
Dinotefuran (375)	80.4 ± 4.8 bc	75.0 ± 2.3 bcd	92.1 ± 1.9 a	83.1 ± 7.3 ab
Clothianidin	67.0 ± 5.8 cd	75.5 ± 4.0 bcd	79.7 ± 5.5 bcd	76.9 ± 7.8 bcd
Bifenthrin	72.5 ± 3.7 cd	77.6 ± 1.1 bcd	91.6 ± 2.2 a	45.5 ± 4.8 e

When analysed by treatment alone (i.e. ignoring time and exposure type) dinotefuran at the high rate was the most toxic insecticide, followed by clothianidin, bifenthrin and low rate dinotefuran. Moderate toxicity was observed with sulfoxaflor and fipronil. Flonicamid and spirotetramat had low toxicity. Cyantraniliprole was not significantly different from the low toxicity insecticides and the moderately toxic fipronil (Figure 20).

In terms of impact on *E. hayati* the ranking of the toxicity of the insecticides are:

- Flonicamid – low
- Spirotetramat – low
- Cyantraniliprole – low
- Sulfoxaflor – moderate
- Fipronil – moderate
- Clothianidin – very high
- Dinotefuran (90) & (375) – very high
- Bifenthrin – very high

While this experiment worked well with the fast acting insecticides, potentially toxic effects of the slower acting products like spirotetramat, cyantraniliprole and flonicamid may have not been fully considered in this bioassay. An experiment investigating sub-lethal effects of these insecticides and others (e.g. pyriproxyfen) may be of value and should be considered for future research.

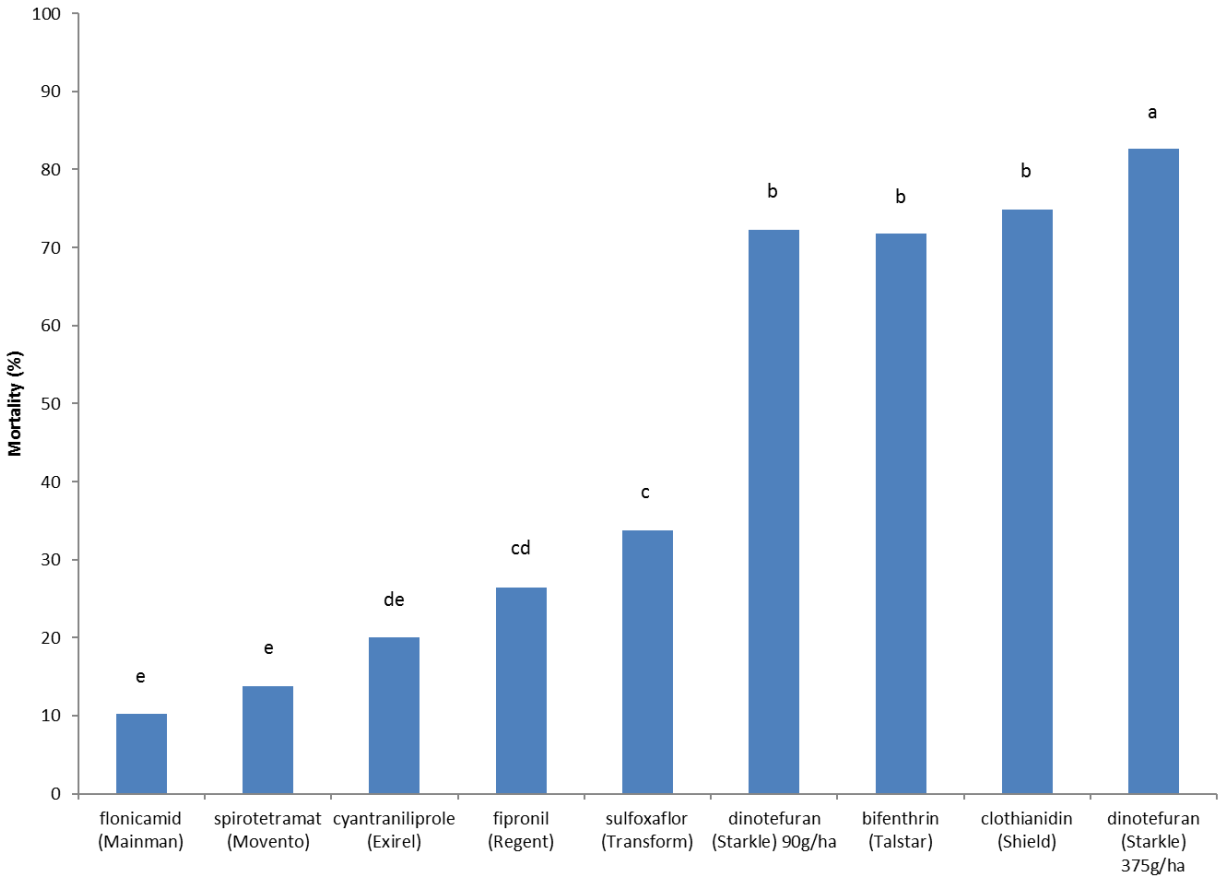

Figure 20. Mean toxicity of insecticides tested against *Eretmocerus hayati*. Columns sharing the same letter are not significantly (P<0.05) different.

4. Predator studies

Prey consumption studies

Rates of prey consumption and development of three natural enemies of cotton aphid (*A. gossypii*) were studied.

Minute two-spotted ladybird beetle

At 25°C, eggs of *D. notescens* took an average (± se) of 5 ± 0.1 days to hatch, larval development took 11 ± 0.4 days, and pupal development took 5 ± 0.3 days (Table 6). To complete larval development, *D. notescens* consumed a mean of 129 ± 5.2 aphids. Consumption of aphids steadily increased with each successive instar. An approximate 24 hour period at the end of the 4th instar was spent searching for a location for pupation; during this period aphid consumption ceased.

During the 54 days of observation, the mean daily aphid consumption of adult *D. notescens* (*n*=20) was 28 ± 1.8 aphids. Female *D. notescens* consumed a daily mean of 34 ± 1.7 aphids, significantly ($F_{1,19}$=34.11, *P*= <0.001) more than males, which consumed 21 ± 1.15 aphids per day.

Average lifespan (*n*=17) of *D. notescens* was 77 ± 9.6 d (range 7–145), with average male lifespan 79 ± 26.7 d and females 75 ± 11.3 d. After eclosion there was a 4-5 day pre-oviposition period before egg lay commenced. Average fecundity (*n*=9) over the life of *D. notescens* was 581 ± 89 eggs with a daily average of 8 ± 0.5 eggs. Female survival rate (l_x) and daily fecundity rate (m_x) were calculated (Figure 21). From those calculations a net reproductive rate (R_o) was estimated as 187 ± 25.1 assuming a 1:1 sex ratio. The intrinsic rate of increase (r_m) was estimated as 0.14 ± 0.002 females/female/day.

Table 6. Mean development (d) and number of aphids ± SE eaten at 25°C for each instar of *Diomus notescens*.

Stage	Development (days)	No. of aphids eaten
egg	5.0 ± 0.1	
1st instar	2.6 ± 0.2	4.8 ± 1.4
2nd instar	1.5 ± 0.2	11.5 ± 1.8
3rd instar	1.7 ± 0.14	23.2 ± 1.6
4th instar	5.3 ± 0.3	91.0 ± 5.3
Total larval	11.1 ± 0.4	129.0 ± 5.2
pupa	5.3 ± 0.3	

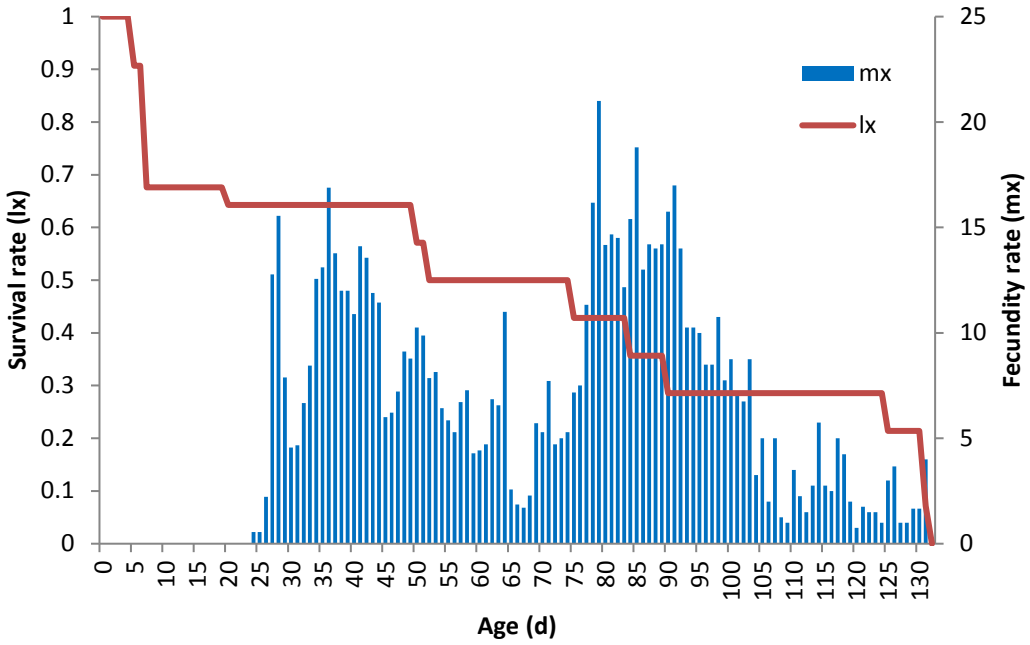

Figure 21. Age specific survival (lx=proportion alive) and fecundity (mx=number of eggs/ female) of *Diomus notescens* (includes juvenile development and mortality).

Transverse ladybird beetle

Total development of transverse ladybeetles required 20 days at 25°C and during larval development they consume an average of 448 aphids (Table 7 & Figure 22).

Table 7. Transverse ladybeetle development and prey consumption of cotton aphids.

Stage	Development (days)	Prey consumption
egg	3.6 ± 0.1 (n=54)	
1st	1.6 ± 0.1 (n= 40)	6.1 ± 0.8 (n=40)
2nd	1.7 ± 0.1 (n=34)	22.5 ±2.5 (n=34)
3rd	2.2 ± 0.1 (n=33)	77.2 ± 6.5 (n=33)
4th	6.1 ± 0.3 (n=12)	341.9 ± 25.0 (n=12)
pupa	4.6 ± 0.2 (n=5)	

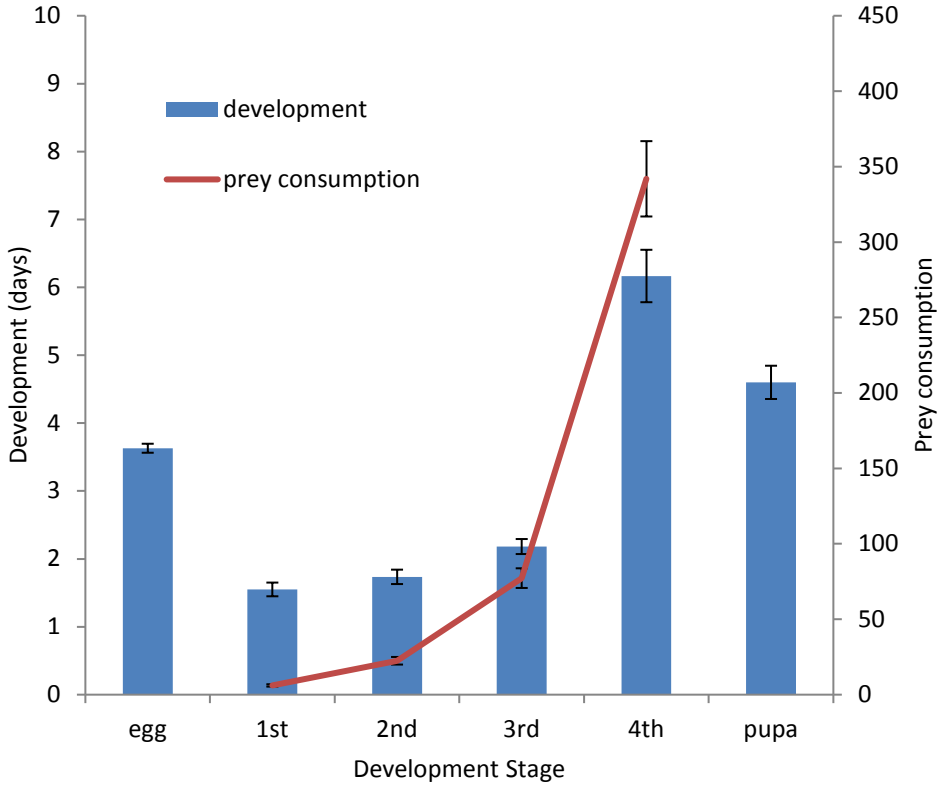

Figure 22. Transverse ladybeetle development and prey consumption of cotton aphids.

Green lacewing

Three prey consumption experiments were conducted with *M. signatus*. The first experiment was on cotton aphid alone (Table 8), the second compared whitefly and cotton aphid (Table 9). The third experiment studied consumption of solenopsis mealybug *P. solenopsis* (Table 10).

Table 8. *Mallada signatus* development and prey consumption of cotton aphids. Number in parentheses is the number of individuals tested.

Stage	Development (days)	No. of aphids eaten
eggs	4.9 ± 0.1 (12)	
1st instar	2.1 ± 0.2 (10)	19 ± 2.3
2nd instar	2.8 ± 0.1 (10)	50 ± 3.6
3rd instar	7.0 ± 0.3 (9)	296 ± 15.8
Pupa	8.4 ± 0.4 (7)	
Total	25.1 ± 0.4 (7)	364 ± 15.2

Prey type offered had no significant effect on larval or pupal development times of *M. signatus*. Between the two prey types there was a significant difference in the number of prey consumed by 3rd instar lacewing larva, with more whitefly nymphs being consumed compared to aphid nymphs.

Table 9. *Mallada signatus* development and prey consumption of *Bemisia tabaci* and *Aphis gossypii.*

Stage	*B. tabaci* as prey		*A. gossypii* as prey	
	development	consumption	development	consumption
i	2.3 ± 0.2	13.3 ± 1.0 d	2.2 ± 0.1	11.5 ± 0.8 d
ii	2.4 ± 0.1	59.1 ± 4.6 c	2.6 ± 0.1	49 ± 3.2 c
iii	4.6 ± 0.3	431.1 ± 23.3 a	5.1 ± 0.3	315.8 ± 15.4 b
pupa	9.3 ± 0.2		8.8 ± 0.2	
total	17.7 ± 0.8	503.1 ± 23.5	18.3 ± 0.4	375.8 ± 14.8

Table 10. *Mallada signatus* development and prey consumption of *Phenaccocus solenopis*

Stage	Development	Prey consumption
i	3.4 ± 0.4 (15)	24.4 ± 3.4 (15)
ii	2.9 ± 0.2 (12)	66.8 ± 9.2 (11)
iii	6.0 ± 0.3 (9)	342.7 ± 12.9 (9)
pupa	9.6 ± 0.3 (8)	
total	21.1 ± 0.6 (8)	426.1 ± 12.3 (9)

Adult lifespan of *M. signatus* was a mean of 21 ± 1.8 d (10 – 42 n=23) for males and 33 ± 2.8d (7 – 58 n=27) for females. Adult lacewings emerge from pupation reproductively immature and females have a pre-oviposition period with a mean of 12 ± 0.7 days (7 – 18 n=22). Mean fecundity was 152 ± 22.1 eggs (5 – 364 n=22) (Figure 23).

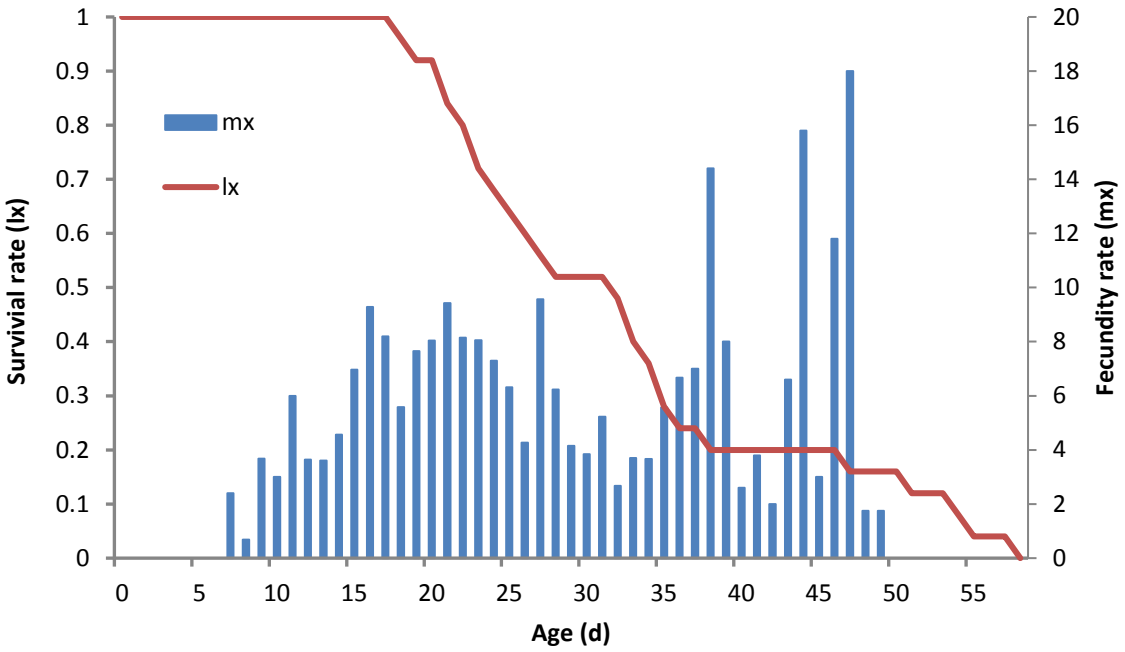

Figure 23. Age specific survival (lx=proportion alive) and fecundity (mx=number of eggs/ female) of adult *Mallada signatus.*

Prey choice studies

Prey selection when offered different prey species was studied for three predators: minute two spotted ladybird, transverse ladybird and green lacewing.

Minute two-spotted ladybird beetle

Two aspects of minute two-spotted ladybird beetle prey preference were studied; preference for particular development stages of whitefly (eggs and nymphs) and for preference of prey species, whitefly eggs and cotton aphid nymphs.

Prey preference for silverleaf whitefly developmental stage

Diomus notescens adults preyed on more whitefly eggs compared to 2nd instar whitefly nymphs or adults; after 24 hours a mean of 19 out of 21 available eggs were eaten compared to 2 out of 21 2nd instar nymphs and 0 out of 21 adults. In a second experiment there was no difference in the predation rates of 1st instar (mean of 6.6 out of 10) and 4th instar whitefly nymphs (mean of 6.1 out of 10). It was observed that eggs were much easier for *D. notescens* to prey on; nymphs being dorso-ventrally flattened appeared to make it much harder for the beetles to attack.

Prey preference for silverleaf whitefly eggs and cotton aphid nymphs

During the preference study investigating species choice, *D. notescens* rarely fed on whitefly eggs when aphid nymphs were present (Table 11). The Manly index of preference for cotton aphid was above 0.9 at each ratio indicating *D. notescens* will more frequently choose cotton aphid nymphs as prey items compared to whitefly eggs (Figure 24). At each prey ratio, preference for aphids was significant, 20:60 ($F_{1,19}$=1398.57, P= <0.001), 40:40 ($F_{1,19}$=3498.68, P= <0.001) and 60:20 ($F_{1,19}$=2.6E+05, P= <0.001).

Prey switching in response to the most abundant prey did not occur; instead *D. notescens* preyed on *A. gossypii* nymphs regardless of prey ratio. At each prey complex ratio, the β value was significantly different from the expected β value; at the 20:60 ratio of whitefly to cotton aphid (SLW t=-26.44, df = 9. P = < 0.001), 40:40 ratio (SLW t=-41.83, df = 9. P = < 0.001) and at 60:20 (SLW t=-362.17, df = 9. P = < 0.001).

In an un-replicated glasshouse experiment where reproductively active *D. notescens* were added to a cage containing a whitefly population, no reproduction was observed over a month long period. This would indicate the whitefly cannot be considered essential prey, but rather should be viewed as alternative prey that can provide maintenance in the absence of other more suitable prey.

The prey choice of *D. notescens* larvae wasn't studied, and it's possible that due to their different mouthpart structure their prey handling may be different to adults.

Table 11. Mean number of prey eaten (± SE) by adult *Diomus notescens* on nymphs of *Aphis gossypii* and eggs of *Bemisia tabaci* when offered at different ratios, and the corresponding preference indices (β).

Prey ratio (whitefly: cotton aphid)	*A. gossypii* consumed	*B. tabaci* consumed	Preference index for *A. gossypii* (β)
0:80	23.1 ± 4.1		
20:60	20.6 ± 1.8	0.4 ± 0.2	0.96
40:40	19.0 ± 1.2	0.4 ± 0.2	0.98
60:20	11.5 ± 1.2	0.1 ± 0.1	0.99
80:0		14.4 ± 7.8	

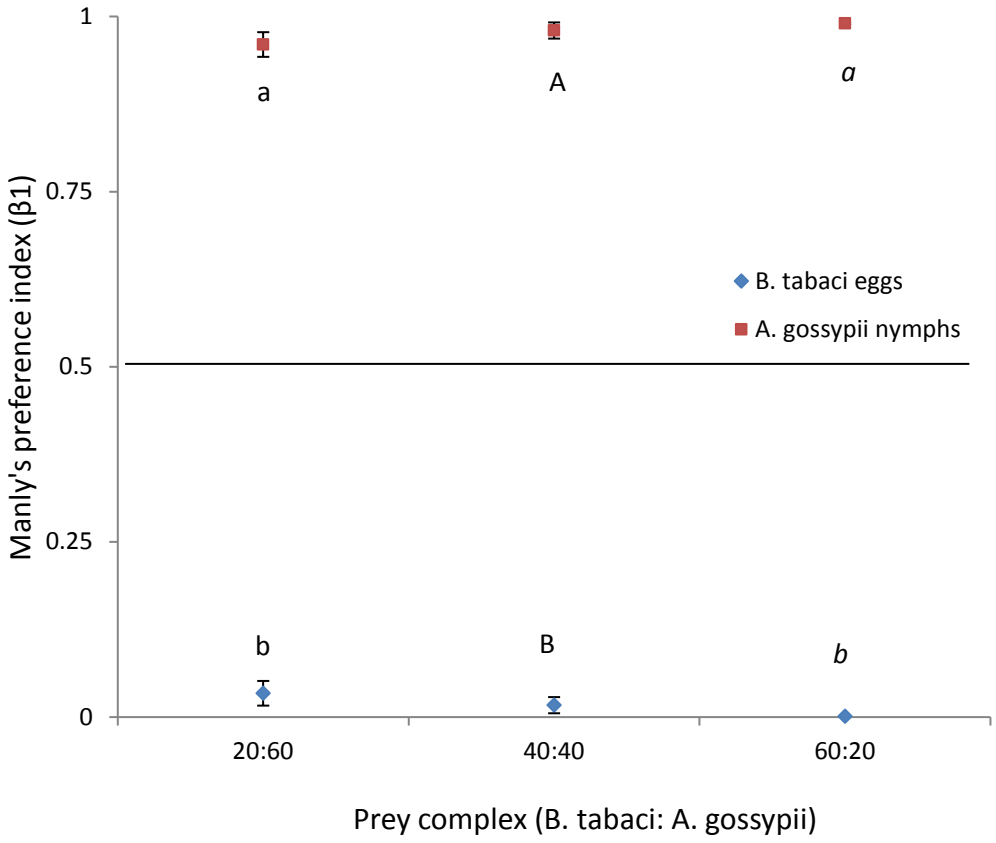

Figure 24. Mean preference **(**± SE) of minute two-spotted ladybird adults for prey (whitefly and cotton aphid) at different initial prey ratios. The solid line represents the expected preference value against which calculated values are compared.

Transverse ladybird beetle

The prey choice of adult transverse ladybeetles was studied in a laboratory experiment by offering beetles different densities of two prey; nymphs of cotton aphid and solenopsis mealybug, *Phenacoccus solenopsis*. Prey preference was calculated using Manly's preference index. The preference index for a given prey type will fall between 0 and 1, a value close to 1 indicates a strong preference for that prey, a value close to 0.5 indicates the predator select prey randomly and a value close to 0 indicates preference for the alternative prey type.

Overall transverse ladybeetles consumed more cotton aphids than solenopsis mealybugs and showed a preference for cotton aphid at each prey ratio, but it wasn't as strong as in the minute two-spotted ladybird above (Table 12 & Figure 25).

Table 12. Number of prey eaten by Transverse ladybeetle and their preference index for cotton aphid

Prey ratio (mealybug: aphid)	*P. solenopsis* consumed	*A. gossypii* consumed	preference index (*A. gossypii*)
0:20		13.5 ± 1.5	
5:15	1.9 ± 0.5	9.2 ± 1.7	0.65 ± 0.08
10:10	4.4 ± 0.8	6.1 ± 0.9	0.63 ± 0.06
15:5	5.0 ± 1.2	3.0 ± 0.6	0.68 ± 0.09
20:0	5.4 ± 1.6		

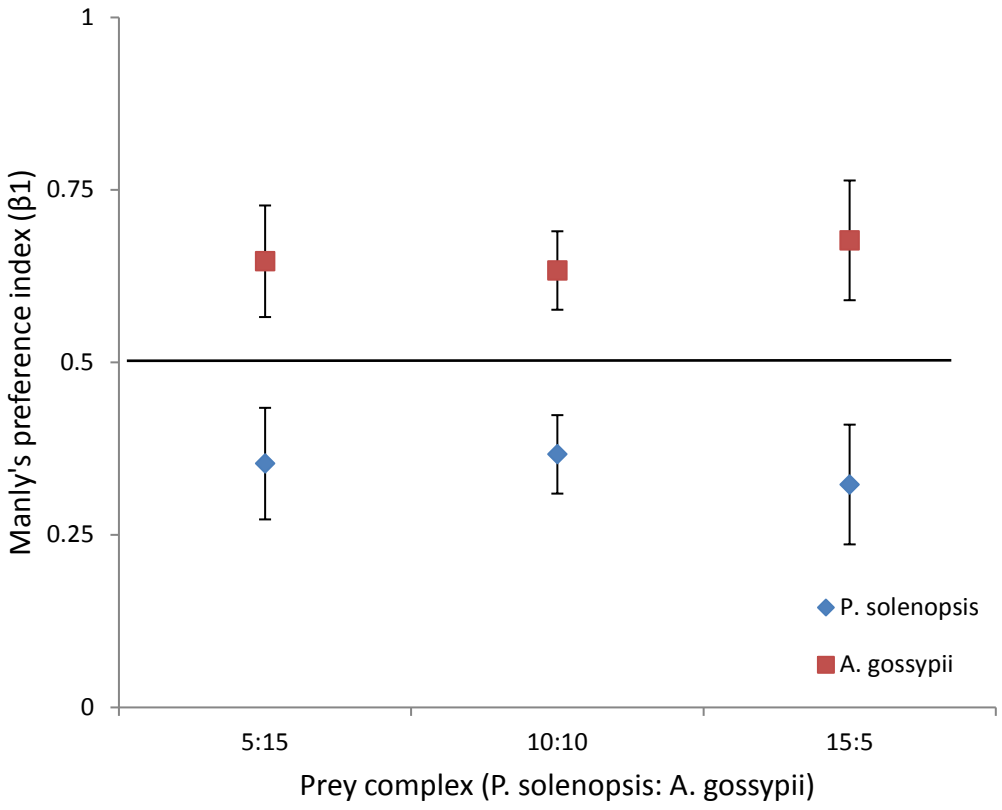

Figure 25. Mean preference **(**± SE) of transverse ladybird adults for prey (solenopsis mealybug and cotton aphid) at different initial prey ratios. The solid line represents the expected preference value against which calculated values are compared.

Green lacewing

With green lacewing the preference of larvae for cotton aphid and silverleaf whitefly was studied using nymphal stages of both pests. At each prey density, *M. signatus* consumed larger proportions of the available *A. gossypii* compared to *B. tabaci* (Table 13). The Manly preference index (β1) for *A. gossypii* at each prey density was significantly ($F_{5,59}$ = 18.21, *P= <0.001*) higher then *B. tabaci.* This would indicate *M. signatus* has a preference for, or more readily detects *A. gossypii* (Figure 26). There was no significant difference in the β1 across the different treatment densities. *Mallada signatus* did consume large numbers of *Bemisia tabaci* at the high density and when offered alone, indicating while they may not be preferred they are still a valued prey item.

There was no evidence for prey switching in response to prey density, at each density the β value was significantly different from the expected value.

Where there was no choice, both pests were readily consumed, suggesting they are both valuable prey for green lacewings.

Table 13. Number of each prey eaten by *Mallada signatus* and preference index for *Aphis gossypii,* value in parentheses is the percentage of total prey available that was consumed.

Prey ratio (whitefly: aphid)	*B. tabaci* consumed	*A. gossypii* consumed	Manly's preference index (β1) (*A. gossypii*)
0:100		54.9 ± 5.3 (54.9%)	
25:75	6.7 ± 2.2 (26.8%)	42.6 ± 6.2 (56.8%)	0.75 ± 0.08
50:50	10.2 ± 3.4 (20.4%)	24.5 ± 3.8 (49.0%)	0.80 ± 0.06
75:25	50.0 ± 5.9 (69.4%)	20.4 ± 1.4 (81.6%)	0.64 ± 0.04
100:0	46.6 ± 8.6 (46.6%)		

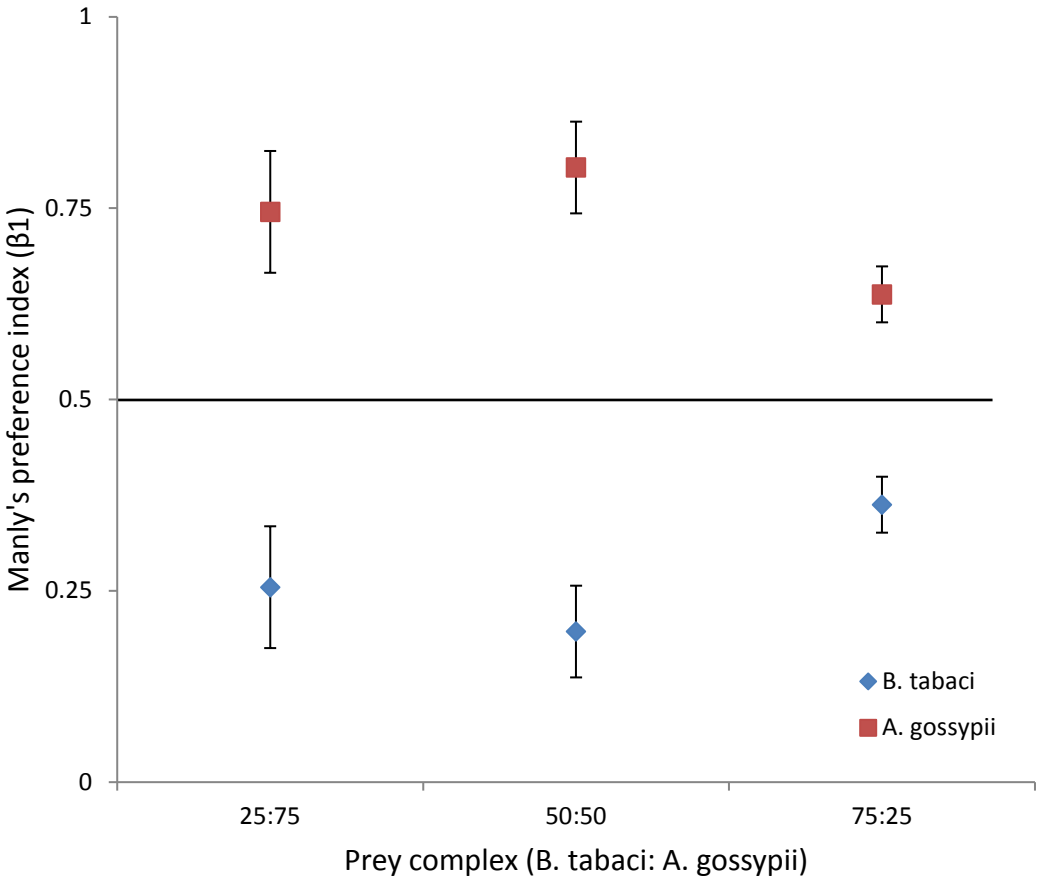

Figure 26. Mean preference **(**± SE) of *Mallada signatus* for prey (whitefly and cotton aphid) at different initial prey ratios. The solid line represents the expected preference value against which calculated values are compared.

Appendix 1. Pyriproxyfen lethal concentration values (LC_{50} & LC_{90})

Locality	Strain	Year	LC_{50} (PPM)	LC_{90} (PPM)	Slope	MEC	RF (LC_{50})	RF (LC_{90})
Lab Susceptible	Susceptible	2014	0.037 (0.026 - 0.052)	0.173 (0.113 - 0.336)	1.91	0.3		
	susceptible	2016	0.019 (0.014 - 0.024)	0.057 (0.041 - 0.094)	2.64	0.1		
St George	SG14-1	2014	0.032 (0.02 - 0.052)	0.21 (0.112 - 0.616)	1.57		1.7	3.7
	SG14-2	2014	0.037 (0.03 - 0.047)	0.126 (0.091 - 0.202)	2.42		1.9	2.2
	SG14-3	2014	0.036 (0.024 - 0.056)	0.236 (0.132 - 0.605)	1.57		1.9	4.2
Emerald	EM14-1	2014	0.017 (0.014 - 0.022)	0.05 (0.037 - 0.076)	2.81	1	0.9	0.9
	EM14-2	2014	0.016 (0.012 - 0.022)	0.112 (0.071 - 0.22)	1.51	1	0.8	2.0
Theodore	TH14-1	2014	0.016 (0.012 - 0.023)	0.069 (0.044 - 0.144)	2.04	1	0.8	1.2
Macintyre	MA14-1	2014	0.045 (0.031 - 0.068)	0.778 (0.403 - 2.058)	1.04		2.4	13.7
	MA14-2	2014	0.021 (0.013 - 0.032)	0.207 (0.107 - 0.643)	1.28		1.1	3.6
	MA14-3	2014	0.046 (0.032 - 0.069)	0.614 (0.329 - 1.56)	1.14		2.4	10.8
Moree	MO14-1	2014	0.025 (0.017 - 0.036)	0.165 (0.097 - 0.387)	1.55		1.3	2.9
	MO14-2	2014	0.027 (0.017 - 0.042)	0.288 (0.151 - 0.82)	1.24		1.4	5.1
Namoi	NM14-1	2014	0.011 (0.007 - 0.016)	0.167 (0.094 - 0.406)	1.07		0.6	2.9
	NM14-2	2014	0.004 (0.002 - 0.006)	0.037 (0.023 - 0.081)	1.35		0.2	0.6
Emerald	EM15-1	2015	0.024 (0.017 - 0.033)	0.15 (0.098 - 0.266)	1.62	2	1.3	2.6
	EM15-2	2015	0.021 (0.012 - 0.035)	0.202 (0.106 - 0.526)	1.29	2	1.1	3.6
St George	SG15-1	2015	0.114 (0.076 - 0.171)	1.726 (1.01 - 3.5)	1.09		6.0	30.4
	SG15-2	2015	0.047 (0.031 - 0.069)	0.548 (0.337 - 1.022)	1.20	2	2.5	9.6
	SG15-3	2015	0.048 (0.035 - 0.064)	0.278 (0.191 - 0.454)	1.68	2	2.5	4.9
Griffith	GR15-1	2015	0.023 (0.014 - 0.037)	0.426 (0.232 - 0.963)	1.01	2	1.2	7.5
Theodore	TH15-1	2015	0.005 (0.003 - 0.007)	0.025 (0.015 - 0.06)	1.77	2	0.3	0.4
Moree	MO15-1	2015	0.022 (0.012 - 0.037)	0.989 (0.483 - 2.651)	0.78		1.2	17.4
	MO15-2	2015	0.02 (0.009 - 0.038)	0.651 (0.278 - 2.338)	0.84		1.1	11.5
Namoi	NM15-1	2015	0.098 (0.066 - 0.141)	0.874 (0.553 - 1.591)	1.35		5.2	15.4
	NM15-2	2015	0.324 (0.219 - 0.478)	4.18 (2.385 - 9.231)	1.15		17.1	73.6
Darling Downs	DD15-1	2015	0.032 (0.021 - 0.048)	0.301 (0.182 - 0.586)	1.32	2	1.7	5.3
Macintyre	MA15-1	2015	0.079 (0.052 - 0.118)	0.986 (0.587 - 1.96)	1.17	2	4.2	17.4
	MA15-2	2015	0.036 (0.022 - 0.057)	0.454 (0.256 - 0.988)	1.16		1.9	8.0
	MA15-3	2015	0.074 (0.049 - 0.109)	0.839 (0.529 - 1.514)	1.22	2	3.9	14.8
Moree	MO16-1	2016	0.033 (0.02 - 0.053)	1.093 (0.554 - 2.761)	0.84	10	1.7	19.2
	MO16-2	2016	0.058 (0.027 - 0.119)	4.16 (1.467 - 20.47)	0.69	10	3.1	73.2
Croppa Creek	Croppa Creek	2016	1.268 (0.495 - 5.12)	48.42 (9.741 - 1743)	0.81		66.7	852.5
	Croppa Creek (2)	2016	0.517 (0.311 - 0.886)	8.812 (4.208 – 25.95)	1.04	10	27.2	154.6
Emerald	EM16-1	2016	0.004 (0.002 - 0.005)	0.015 (0.009 - 0.032)	2.05	10	0.2	0.3
Theodore	TH16-1	2016	0.040 (0.033 - 0.048)	0.012 (0.008 - 0.022)	3.40	10	2.2	1.7
	TH16-2	2016	0.038 (0.032 - 0.046)	0.015 (0.009 - 0.031)	3.51	1	2.1	1.6
Macintyre	MA16-1	2016	0.009 (0.004 - 0.017)	0.797 (0.321 - 3.052)	0.65	10	0.5	14.0
	MA16-2	2016	0.2183 (0.1257 - 0.3771)	1.323 (0.695 - 3.98)	1.64	10	11.5	23.3
	MA16-3	2016	0.038 (0.022 - 0.065)	0.951 (0.464 - 2.587)	0.92	10	2.0	16.7
St George	SG16-1	2016	0.076 (0.047 - 0.124)	2.354 (1.184 - 5.921)	0.86	10	4.0	41.4
	SG16-2	2016	0.099 (0.062 - 0.158)	2.2 (1.144 - 5.329)	0.95	10	5.2	38.7
	SG16-3	2016	0.086 (0.045 - 0.162)	1.514 (0.675 - 5.172)	1.03	10	4.5	26.7
Namoi	NM16-1	2016	0.095 (0.073 - 0.122)	1.009 (0.708 - 1.541)	1.25	10	5.0	17.8
	NM16-2	2016	0.1 (0.064 - 0.154)	1.213 (0.684 - 2.65)	1.18		5.3	21.4

Locality	Strain	Year	LC_{50} (PPM)	LC_{90} (PPM)	Slope	MEC	RF (LC_{50})	RF (LC_{90})
Boomi	MA16-4	2016	0.098 (0.074 - 0.131)	2.432 (1.587 - 4.059)	0.92	10	5.2	42.8
Mungindi	MU16-1	2016	0.282 (0.167 - 0.485)	7.162 (3.336 - 21.06)	0.91	10	14.8	126.1
Hillston	HI16-1	2016	0.021 (0.014 - 0.032)	0.212 (0.124 - 0.446)	1.29	10	1.1	3.7

Appendix 2. Diafenthiuron lethal concentration values (LC_{50} & LC_{90})

Locality	Strain	Year	LC_{50} (PPM)	LC_{90} (PPM)	Slope	MEC	RF (LC_{50})	RF (LC_{90})
Lab	Susceptible	2014	33.36 (27.05 - 41.11)	76.99 (59.55 - 114.5)	3.5	300		
Namoi	NM14-1	2014	13.52 (10.36 - 16.97)	28.29 (21.61 - 48.14)	4.0	100	0.4	0.4
	NM14-2	2014	16.79 (14.14 - 19.81)	28.18 (23.45 - 37.11)	5.7	100	0.5	0.4
Macintyre	MA14-1	2014	43.37 (34.35 - 55.65)	84.85 (64.29 - 140.8)	4.4	300	1.3	1.1
	MA14-2	2014	43.48 (26.63 - 73.96)	96.85 (60.78 - 385.3)	3.7	100	1.3	1.3
	MA14-3	2014	44.2 (38.19 - 51.14)	95.28 (79.21 - 122.4)	3.8	300	1.3	1.2
Moree	MO14-1	2014	15.72 (11.06 - 21.32)	38.5 (27.04 - 82.92)	3.3	100	0.5	0.5
	MO14-2	2014	7.222 (0.5542 - 10.4)	16.68 (11.83 - 94.64)	3.5	30	0.2	0.2
St George	SG14-1	2014	40.91 (32.22 - 52.04)	108 (80.12 - 171.4)	3.0	300	1.2	1.4
	SG14-2	2014	43.92 (33.8 - 56.86)	131.9 (95.3 - 217.9)	2.7	300	1.3	1.7
	SG14-3	2014	28.31 (19.67 - 39.37)	95.94 (64.33 - 190.6)	2.4	300	0.8	1.2
Emerald	EM14-1	2014	37.6 (17.74 - 83.55)	101 (54.64 - 1438)	3.0	100	1.1	1.3
	EM14-2	2014	13.5 (8.513 - 18.59)	35.87 (24.75 - 85.95)	3.0	100	0.4	0.5
Theodore	TH14-1	2014	17.16 (8.649 - 27.45)	56.69 (33.85 - 229.5)	2.5	100	0.5	0.7
Emerald	EM15-1	2015	14.57 (9.244 - 22.04)	25.3 (18.23 - 134.7)	5.4	50	0.4	0.3
	EM15-2	2015	13.61 (7.562 - 19.32)	36.78 (24.78 - 106.4)	3.0	100	0.4	0.5
St George	SG15-1	2015	11.17 (2.141 - 16.5)	23.7 (16.16 - 863.9)	3.9	50	0.3	0.3
	SG15-2	2015	17.45 (14.33 - 20.87)	41.79 (32.97 - 61.12)	3.4	300	0.5	0.5
	SG15-3	2015	13.55 (11.54 - 15.57)	21.25 (18.08 - 28.64)	6.6	50	0.4	0.3
Theodore	TH15-1	2015	10.68 (7.72 -12.94)	22.94 (18.21 - 37.98)	3.9	50	0.3	0.3
Moree	MO15-1	2015	26.96 (21.33 - 34.24)	64.01 (47.83 - 103.6)	3.4	300	0.8	0.8
	MO15-2	2015	35.5 (26.37 - 47.5)	88.26 (62.62 - 168.3)	3.2	100	1.1	1.1
Namoi	NM15-1	2015	15.74 (11.55 - 21.32)	29.2 (21.5 - 71.15)	4.8	50	0.5	0.4
	NM15-2	2015	14.97 (10.19 - 20.38)	32.03 (22.8 - 85.31)	3.9	50	0.4	0.4
Darling Downs	DD15-1	2015	9.564 (4.192 - 13.91)	34.34 (23.57 - 79.14)	2.3	300	0.3	0.4
Macintyre	MA15-1	2015	14.38 (7.965 - 20.84)	43.25 (28.34 - 124.3)	2.7	100	0.4	0.6
	MA15-2	2015	18.78 (11.5 - 26.71)	88.57 (57.29 - 197.3)	1.9	300	0.6	1.2
	MA15-3	2015	13.22 (9.06 - 17.05)	31.48 (23.2 - 63.39)	3.4	100	0.4	0.4
Griffith	GR15-1	2015	17.38 (14.46 - 21.12)	31.92 (25.27 - 50.19)	4.9	100	0.5	0.4
St George	SG16-1	2016	21.18 (18.47 - 24.39)	42.98 (35.73 - 55.96)	4.2	100	0.6	0.6
	SG16-2	2016	18.49 (14.6 - 23.63)	42.46 (31.58 - 70.72)	3.5	100	0.6	0.6
	SG16-3	2016	13.91 (10.54 - 17.38)	28.93 (22.62 - 43.28)	4.0	100	0.4	0.4
Moree	MO16-1	2016	20.51 (15.22 - 27.91)	57.58 (39.85 - 107.9)	2.9	300	0.6	0.7
	MO16-2	2016	23 (14.39 - 38.14)	74.08 (43.28 - 240.2)	2.5	100	0.7	1.0
Macintyre	MA16-1	2016	33.29 (26.43 - 42.36)	83.65 (61.98 - 135.7)	3.2	100	1.0	1.1
	MA16-2	2016	25.53 (18.7 - 34.81)	45.03 (33.46 - 112.7)	5.2	100	0.8	0.6
	MA16-3	2016	29.31 (17.58 - 52.93)	90.68 (50.82 - 374.3)	2.6	100	0.9	1.2
Emerald	EM16-1	2016	19.81 (15.64 - 25.36)	43.72 (32.61 - 73.49)	3.7	100	0.6	0.6
Theodore	TH16-1	2016	16.69 (13.41 - 20.82)	40.88 (30.96 - 63.45)	3.3	100	0.5	0.5

Locality	Strain	Year	LC_{50} (PPM)	LC_{90} (PPM)	Slope	MEC	RF (LC_{50})	RF (LC_{90})
	TH16-2	2016	37.77 (33.48 - 43.57)	65.15 (54.13 - 89.02)	5.4	100	1.1	0.8
Hillston	HI16-1	2016	4.21(1.97 -6.55)	12.65 (7.871 - 47.88)	2.7	30	0.1	0.2

Appendix 3. Spirotetramat lethal concentration values (LC_{50} & LC_{90})

Locality	Strain	Year	LC_{50} (PPM)	LC_{90} (PPM)	Slope	MEC	RF (LC_{50})	RF (LC_{90})
Lab	susceptible	2014	6 (4.9 - 7.4)	20.9 (15.5 – 34.2)	2.379	30		
Emerald	EM14-1	2014	11.5 (10 - 13.5)	28.2 (22.2 - 38.5)	3.337		1.9	1.3
	EM14-2	2014	11.2 (9 - 14.5)	25.2 (18.4 - 44.4)	3.624		1.9	1.2
Theodore	TH14-1	2014	9.3 (7.3 - 12.5)	39.1 (25.6 - 78.5)	2.063		1.6	1.9
St George	SG14-1	2014	6.2 (5 - 7.7)	20.2 (14.8 – 32.8)	2.494		1.0	1.0
	SG14-2	2014	5 (4.1 - 5.9)	12.4 (9.9 - 17.4)	3.232	30	0.8	0.6
	SG14-3	2014	5.8 (5 - 6.7)	16.2 (13 - 22.1)	2.874		1.0	0.8
Moree	MO14-1	2014	6.4 (5.6 - 7.4)	24.9 (20 - 32.9)	2.179		1.1	1.2
	MO14-2	2014	6.2 (4 - 10.1)	30.1 (16.1 - 127.8)	1.865		1.0	1.4
Namoi	NM14-1	2014	11.6 (9.3 - 14.9)	36.6 (25.6 - 65.5)	2.558		1.9	1.8
	NM14-2	2014	8.3 (6.9 - 10.2)	18.4 (14.6 - 25.3)	2.819		0.9	0.9
Macintyre	MA14-1	2014	7 (5.6 - 8.7)	23.7 (17.8 - 37.1)	3.417		1.4	1.1
	MA14-2	2014	5.6 (4.8 - 6.6)	16.5 (12.3 - 28.4)	2.498		1.2	0.8
	MA14-3	2014	3.9 (3.2 - 4.8)	12.3 (9.4 - 18.3)	2.589		0.7	0.6
Emerald	EM15-1	2015	9.9 (7.6 - 12.4)	41.6 (32.8 - 55.8)	2.057	100	1.7	2.0
	EM15-2	2015	9.4 (8.1 - 10.8)	42.9 (36.2 - 52.6)	1.943		1.6	2.1
Theodore	TH15-1	2015	8.5 (6.1 - 11.1)	29.6 (22 - 44.9)	2.369	56	1.4	1.4
St George	SG15-1	2015	7.6 (5.7 - 9.8)	35.1 (26.8 - 48.9)	1.932	100	1.3	1.7
	SG15-2	2015	9.7 (6.7 - 13)	49.4 (35.5 - 77.1)	1.811		1.6	2.4
	SG15-3	2015	10.8 (8 - 13.7)	40.4 (30.5 - 60.2)	2.232	56	1.8	1.9
Moree	MO15-1	2015	7.9 (5.4 - 10.3)	23.4 (17.7 - 34.5)	2.705	100	1.3	1.1
	MO15-2	2015	5.3 (3.3 - 7.9)	40.7 (26.7 - 72.3)	1.454		0.9	1.9
Namoi	NM15-1	2015	5.1 (4.3 - 6.1)	15.6 (13.1 - 19)	2.654	56	0.9	0.7
	NM15-2	2015	5.1 (2.7 - 8.4)	17.7 (10.6 - 42.8)	2.366	30	0.9	0.8
Darling Downs	DD15-1	2015	6.2 (5 - 7.5)	39.7 (32 - 51)	1.586	100	1.0	1.9
Macintyre	MA15-1	2015	6.8 (4.9 - 9)	33.3 (24.7 - 48.3)	1.858		1.1	1.6
	MA15-2	2015	6.8 (4.4 - 9.7)	53.5 (36.6 - 88.8)	1.434		1.1	2.6
	MA15-2	2015	2.3 (1.4 - 3.3)	14.9 (9.9 - 26.7)	1.569	30	0.4	0.7
	MA15-3	2015	2.8 (2.2 - 3.5)	9.4 (7.2 - 13.4)	2.43	30	0.5	0.4
Griffith	GR15-1	2015	6 (4.9 - 7.1)	17 (14.3 - 20.8)	2.824	56	1.0	0.8
Emerald	EM16-1	2016	6.8 (5.2 - 9.1)	28 (18.6 - 52.5)	2.083	100	1.1	0.4
Theodore	TH16-1	2016	5.7 (3.4 - 8.8)	67.9 (38 - 165.7)	1.187	300	1.0	3.2
St George	SG16-2	2016	12.7 (9.3 - 18.6)	69 (41.4 - 150)	1.748	100	2.1	3.3
Moree	MO16-1	2016	5.0 (3.3 - 7.8)	31.7 (17.2 - 97.3)	1.595	100	0.8	1.5
Namoi	NM16-2	2016	9.9 (8.4 - 11.8)	44.0 (34.1 - 60.12)	1.979	300	1.6	2.1
Macintyre	MA16-3	2016	3.1 (2.1 - 4.3)	14.3 (9 - 33.6)	1.928	100	0.5	0.7
Hillston	HI16-1	2016	5.3 (3.6 - 8.2)	26.5 (14.9 - 79.9)	1.831	100	0.9	1.3

Appendix 4. Cyantraniliprole lethal concentration values (LC_{50} & LC_{90})

Locality	Strain	Year	LC_{50} (PPM)	LC_{90} (PPM)	Slope	MEC	RF (LC_{50})	RF (LC_{90})
Lab	Susceptible	2014	0.01 (0.008 - 0.012)	0.035 (0.028 - 0.048)	2.325	0.2		
	Susceptible	2016	0.078 (0.065 - 0.094)	0.112 (0.093 - 0.141)	3.306	0.3		
Emerald	EM14-1	2014	0.017 (0.013 - 0.023)	0.063 (0.042 - 0.116)	2.289	0.1	1.7	1.8
	EM14-2	2014	0.015 (0.013 - 0.017)	0.042 (0.035 - 0.051)	2.432		1.5	1.2
Theodore	TH14-1	2014	0.012 (0.009 - 0.017)	0.042 (0.029 - 0.077)	2.48		1.3	1.2
Macintyre	MA14-1	2014	0.009 (0.007 - 0.013)	0.052 (0.039 - 0.075)	1.869		1.0	1.5
	MA14-2	2014	0.015 (0.013 - 0.017)	0.052 (0.043 - 0.066)	2.432		1.5	1.5
	MA14-3	2014	0.004 (0.002 - 0.005)	0.015 (0.009 - 0.04)	2.003		0.4	0.4
Moree	MO14-1	2014	0.015 (0.011 - 0.02)	0.063 (0.043 - 0.114)	2.047		1.5	1.8
	MO14-2	2014	0.016 (0.014 - 0.02)	0.061 (0.047 - 0.087)	2.221		1.6	1.7
Namoi	NM14-1	2014	0.015 (0.01 - 0.022)	0.065 (0.041 - 0.14)	2.024		1.5	1.9
	NM14-2	2014	0.018 (0.013 - 0.024)	0.087 (0.058 - 0.162)	1.969		1.9	2.5
St George	SG14-1	2014	0.016 (0.014 - 0.018)	0.031 (0.026 - 0.038)	4.526	0.1	1.6	0.9
	SG14-2	2014	0.008 (0.007 - 0.01)	0.033 (0.033 - 0.033)	2.227		0.9	0.9
	SG14-3	2014	0.037 (0.028 - 0.05)	0.131 (0.088 - 0.247)	2.332		3.7	3.7
Emerald	EM15-1	2015	0.007 (0.002 - 0.013)	0.098 (0.058 - 0.281)	1.12	0.2	0.7	2.8
	EM15-2	2015	0.022 (0.014 - 0.029)	0.073 (0.056 - 0.108)	2.417	0.2	2.2	2.1
Theodore	TH15-1	2015	0.013 (0.009 - 0.018)	0.045 (0.031 - 0.091)	2.436	0.2	1.3	1.3
St George	SG15-1	2015	0.014 (0.011 - 0.017)	0.036 (0.028 - 0.055)	3.109	0.1	1.4	1.0
	SG15-2	2015	0.031 (0.023 - 0.042)	0.072 (0.05 - 0.138)	3.465		3.1	2.1
	SG15-3	2015	0.024 (0.021 - 0.027)	0.064 (0.054 - 0.081)	2.95	0.2	2.4	1.8
Moree	MO15-1	2015	0.031 (0.028 - 0.036)	0.086 (0.072 - 0.109)	2.931	0.2	3.1	2.5
	MO15-2	2015	0.022 (0.018 - 0.026)	0.072 (0.057 - 0.099)	2.496	0.2	2.2	2.1
Namoi	NM15-1	2015	0.019 (0.016 - 0.023)	0.067 (0.054 - 0.087)	2.399	0.2	1.9	1.9
	NM15-2	2015	0.034 (0.027 - 0.042)	0.097 (0.075 - 0.143)	2.792	0.2	3.4	2.8
Darling Downs	DD15-1	2015	0.028 (0.021 - 0.038)	0.105 (0.071 - 0.201)	2.246	0.2	2.8	3.0
Macintyre	MA15-1	2015	0.03 (0.024 - 0.037)	0.07 (0.054 - 0.099)	3.524	0.2	3.0	2.0
	MA15-2	2015	0.039 (0.021 - 0.057)	0.067 (0.047 - 0.164)	5.28	0.2	3.9	1.9
	MA15-3	2015	0.019 (0.015 - 0.024)	0.055 (0.04 - 0.096)	2.813	0.1	1.9	1.6
Griffith	GR15-1	2015	0.036 (0.028 - 0.045)	0.106 (0.078 - 0.171)	2.713		3.6	3.0
Emerald	EM16-1	2016	0.044 (0.035 - 0.058)	0.125 (0.09 - 0.207)	2.858	0.3	0.6	1.1
Theodore	TH16-1	2016	0.026 (0.021 - 0.032)	0.065 (0.05 - 0.097)	3.218	0.3	0.3	0.6
	TH16-2	2016	0.016 (0.013 - 0.02)	0.038 (0.029 - 0.062)	3.496	0.3	0.2	0.3
St George	SG16-1	2016	0.041 (0.035 - 0.05)	0.14 (0.108 - 0.2)	2.425	0.3	0.5	1.3
	SG16-2	2016	0.03 (0.024 - 0.038)	0.073 (0.054 - 0.116)	3.296	0.3	0.4	0.7
	SG16-3	2016	0.025 (0.02 - 0.031)	0.048 (0.037 - 0.076)	4.432	0.3	0.3	0.4
Macintyre	MA16-1	2016	0.009 (0.004 - 0.015)	0.047 (0.03 - 0.115)	1.797	0.3	0.1	0.4
	MA16-2	2016	0.01 (0.007 - 0.012)	0.03 (0.024 - 0.04)	2.614	0.1	0.1	0.3
	MA16-3	2016	0.019 (0.011 - 0.031)	0.063 (0.037 - 0.345)	2.468	0.1	0.2	0.6
	MA16-4	2016	0.023 (0.02 - 0.028)	0.059 (0.045 - 0.088)	3.179	0.3	0.3	0.5
Moree	MO16-1	2016	0.02459 (0.018 - 0.033)	0.103 (0.067 - 0.222)	2.058	0.3	0.3	0.9
	MO16-2	2016	0.03789 (0.028 - 0.05)	0.127 (0.091 - 0.208)	2.438	1.0	0.5	1.1
	Croppa Creek	2016	0.049 (0.042 - 0.056)	0.125 (0.105 - 0.157)	3.115	0.3	0.6	1.1
Namoi	NM16-1	2016	0.017 (0.014 - 021)	0.04 (0.032 - 0.053)	3.624	0.1	0.2	0.4
	NM16-2	2016	0.011 (0.008 - 0.014)	0.03 (0.025 - 0.043)	2.87	0.1	0.1	0.3
Mungindi	MU16-1	2016	0.023 (0.019 - 0.028)	0.061 (0.047 - 0.091)	3.019	0.3	0.3	0.5

Locality	Strain	Year	LC_{50} (PPM)	LC_{90} (PPM)	Slope	MEC	RF (LC_{50})	RF (LC_{90})
Hillston	HI16-1	2016	0.025 (0.015 - 0.042)	0.09 (0.051 - 0.461)	2.318	0.1	0.3	0.8

Appendix 5. Dinotefuran systemic lethal concentration values (LC_{50} & LC_{90})

Locality	Strain	Year	LC_{50} (PPM)	$LC_{99.9}$ (PPM)	Slope	MEC	RF (LC_{50})	RF ($LC_{99.9}$)
Lab	Susceptible	2015	0.15 (0.11 - 0.2)	1.1 (0.6 - 4.4)	3.629	1		
Emerald	EM15-1	2015	0.46 (0.37 - 0.56)	6 (3.6 - 13.3)	2.752	10	3.1	5.5
	EM15-2	2015	0.72 (0.53 - 0.99)	8.8 (4.5 - 31.8)	2.838	10	4.8	8.0
Theodore	TH15-1	2015	0.51 (0.41 - 0.64)	6.3 (3.8 - 14)	2.839	10	3.4	5.7
St George	SG15-1	2015	0.3 (0.11 - 0.58)	116.9 (20.7 - 10453)	1.195	10	2.7	13.6
	SG15-2	2015	0.99 (0.81 - 1.19)	7 (4.6 - 14.3)	3.621	10	6.6	6.4
	SG15-3	2015	0.47 (0.38 - 0.57)	5.5 (3.4 - 11.6)	2.887	3.2	3.1	5.0
Moree	MO15-1	2015	0.46 (0.36 - 0.6)	7.9 (4.3 - 21.7)	2.509	10	3.1	7.2
	MO15-2	2015	0.54 (0.39 - 0.75)	5.8 (2.9 - 25.9)	3.01	3.2	3.6	5.3
Namoi	NM15-1	2015	0.59 (0.44 - 0.78)	25 (12.6 - 70.3)	1.898	10	3.9	22.7
	NM15-2	2015	0.39 (0.26 - 0.56)	13 (5.6 - 62.3)	2.031	10	2.6	11.8
Macintyre	MA15-1	2015	0.97 (0.67 - 1.4)	39.7 (17.1 - 163.2)	1.92	32	6.5	36.1
	MA15-2	2015	0.45 (0.35 - 0.57)	6.5 (3.7 - 16.6)	2.655	3.2	3.0	5.9
	MA15-3	2015	0.6 (0.43 - 0.84)	9.7 (4.6 - 39.9)	2.561	10	4.0	8.8
Darling Downs	DD15-1	2015	0.26 (0.2 - 0.35)	4.2 (2.2 - 13.9)	2.562	3.2	1.7	3.8
Griffith	GR15-1	2015	0.28 (0.23 - 0.33)	8.1 (4.9 - 16.9)	2.106	3.2	1.9	7.4
Gumlu	PA1510-R	2015	1.37 (0.83 - 2.29)	32.2 (11.5 - 367.1)	2.252	10	9.1	29.3
Ayr	AY09-1R	2015	1.27 (0.92 - 1.74)	36.4 (17.3 - 124.9)	2.119	32	8.5	33.1
Gatton	Shield R	2015	1.16 (0.96 - 1.4)	24.6 (15.3 - 47.3)	2.327	32	7.7	22.4

Appendix 6. Dinotefuran foliar lethal concentration values (LC_{50} & LC_{90})

Locality	Strain	Year	LC50 (PPM)	LC 99.9 (PPM)	Slope	MEC	RF 50	RF 99.9
Lab	Susceptible	2015	12.3 (8.3 - 18.2)	1108 (428.4 - 5085)	1.581	320		
Namoi	NM15-1	2015	6.9 (5.5 - 8.6)	97.1 (57.5 - 217.3)	2.69	320	0.6	0.1
	NM15-2	2015	10.5 (7.7 - 14.1)	339.2 (163.9 - 1087)	2.045	100	0.9	0.3
Theodore	TH15-1	2015	8.3 (5.8 - 11.7)	746.3 (314.5 - 2801)	1.581	320	0.7	0.7
Emerald	EM15-1	2015	5.4 (3.2 - 10.1)	464.2 (117.4 - 9007)	1.598	32	0.4	0.4
	EM15-2	2015	3 (1.6 - 5.3)	226 (59.5 - 4759)	1.64	32	0.2	0.2
Griffith	GR15-1	2015	22 (17.7 - 27.2)	267 (160.1 - 598.8)	2.848	320	1.8	0.2
St George	SG15-1	2015	7.9 (6.1 - 10.1)	153.9 (86.4 - 372.2)	2.394	320	0.6	0.1
	SG15-2	2015	5.3 (4.1 - 6.8)	159.4 (84.7 - 411.5)	2.087	320	0.4	0.1
	SG15-3	2015	5.6 (4.1 - 7.7)	465.8 (212.4 - 1474)	1.611	320	0.5	0.4
Moree	MO15-1	2015	7.1 (5.5 - 9.2)	42.6 (25.1 - 128.5)	3.983	32	0.6	0.0
	MO15-2	2015	6.1 (4.6 - 8.3)	86.5 (44.7 - 285.2)	2.691	100	0.5	0.1
Griffith	GR15-1	2015	7.4 (5.7 - 9.5)	242 (126.8 - 635.1)	2.038	100	0.6	0.2
Darling Downs	DD15-1	2015	9.4 (7.4 - 11.9)	144.6 (82 - 354.3)	2.603	100	0.8	0.1
Macintyre	MA15-1	2015	6.6 (4.8 - 9.2)	90.2 (46.6 - 298.1)	2.729	100	0.5	0.1
	MA15-2	2015	5.1 (3.4 - 7.4)	648.1 (254.4 - 2739)	1.467	320	0.4	0.6
	MA15-3	2015	9.9 (7.3 - 13.4)	263.3 (131.6 - 795.6)	2.168		0.8	0.2

Locality	Strain	Year	LC50 (PPM)	LC 99.9 (PPM)	Slope	MEC	RF 50	RF 99.9
Gumlu	PA1510-R	2015	24.3 (17.7 - 33.5)	1019 (470.5 - 3423)	1.904	320	2.0	0.9
Ayr	AY09-1R	2015	17.5 (11.5 - 28)	1235 (403.5 - 9697)	1.672	100	1.4	1.1
Emerald	EM16-1	2016	28 (15.4 - 50.6)	1227 (321 - 67744)	1.883	100	2.3	1.1
Theodore	TH16-1	2016	23.5 (17.4 - 31)	222 (131 - 544)	2.383	320	1.9	0.2
	TH16-2	2016	10.6 (6 - 17.1)	465 (168 - 3872)	1.883	100	0.9	0.4
St George	SG16-1	2016	10.4 (6.7 - 15.1)	1032 (427.5 - 4354)	1.56	320	0.8	0.9
	SG16-2	2016	15.5 (10 - 22.5)	2586 (961.5 - 12836)	1.39	320	1.3	2.3
	SG16-3	2016	6.2 (4.5 - 8.2)	515.1 (272 - 1250)	1.61	100	0.5	0.5
Macintyre	MA16-1	2016	16.1 (10.4 - 23.1)	453 (194 - 2438)	2.131	320	1.3	0.4
	MA16-2	2016	10.8 (7.9 - 14.2)	1184 (604 - 3034)	1.514	320	0.9	1.1
	MA16-3	2016	24.1 (15.9 - 34.6)	1458 (580 - 7523)	1.734	320	2.0	1.3
Moree	MO16-1	2016	5.1 (4.2 – 6.2)	55.4 (39.01 – 90.08)	2.988	32	0.4	0.1
	MO16-2	2016	12.1 (9.3 - 15.2)	504 (279 - 1202)	1.906	100	1.0	0.5
Namoi	NM16-1	2016	6.2 (3.7 - 8.7)	61 (33 - 254)	3.126	32	0.5	0.1
	NM16-2	2016	10.8 (6.7 - 15.8)	440 (181 - 2412)	1.917	100	0.9	0.4
Hillston	HI16-1	2016	5.1 (2.6 - 8.4)	139 (56 - 1014)	2.154	100	0.4	0.1

Appendix 7. Bifenthrin lethal concentration values (LC_{50} & LC_{90})

Locality	Strain	Year	LC_{50} (PPM)	LC_{90} (PPM)	Slope	MEC	RF (LC_{50})	RF (LC_{90})
Lab	Susceptible	2014	3 (2.4 - 3.6)	9.6 (7.4 - 13.6)	2.5	85		
Moree	MO14-1	2014	16.4 (12.2 - 20.7)	83.8 (63.8 - 122.3)	1.8		5.5	8.7
	MO14-2	2014	18.8 (14 - 23.9)	129.9 (95.8 - 197.5)	1.5		6.3	13.5
Namoi	NM14-1	2014	16.4 (11.6 - 21.5)	55.3 (39.4 - 98.5)	2.4		5.5	5.8
	NM14-2	2014	14.7 (11.3 - 18)	59.6 (45.1 - 90.8)	2.1	100	4.9	6.2
St George	SG14-1	2014	16.2 (9.2 - 23.5)	65.8 (42.8 - 149.1)	2.1	300	5.4	6.9
	SG14-2	2014	17.1 (12.5 - 22)	95.1 (69.2 - 150)	1.7		5.7	9.9
	SG14-3	2014	2.7 (0.1 - 7.4)	60.2 (32.2 - 203.2)	1.0	300	0.9	6.3
Macintyre	MA14-1	2014	8.4 (5.3 - 11.6)	54.3 (40.4 - 83.4)	1.6	300	2.8	5.7
	MA14-2	2014	14 (7.1 - 21.3)	62.6 (39.2 - 163.4)	2.0	300	4.7	6.5
	MA14-3	2014	7.3 (4.3 - 10.3)	42.5 (31.8 - 65.4)	1.7		2.4	4.4
Emerald	EM14-1	2014	5.8 (0.7 - 12.9)	101.7 (53.5 - 427.4)	1.0		1.9	10.6
	EM14-2	2014	8.3 (2.1 - 15.3)	75.7 (42.3 - 268.2)	1.3		2.8	7.9
Theodore	TH14-1	2014	9.3 (5.6 - 13.1)	79.2 (58.3 - 123.1)	1.4		3.1	8.3
St George	SG15-1	2015	12.3 (8.1 - 16.8)	99.5 (75.3 - 142.9)	1.4	500	4.1	10.4
	SG15-2	2015	13.6 (8.8 - 18.5)	89.1 (65.7 - 135.1)	1.6	250	4.5	9.3
	SG15-3	2015	10.8 (5.3 - 16.8)	83.1 (57.3 - 141.7)	1.4		3.6	8.7
Theodore	TH15-1	2015	5 (1.8 - 8.1)	28.9 (21.7 - 43.7)	1.7	125	1.7	3.0
Griffith	GR15-1	2015	15.1 (11.4 - 18.8)	67.1 (52.4 - 95)	2.0	250	5.0	7.0
Emerald	EM15-1	2015	12.9 (10.1 - 16.3)	57.5 (43.8 - 80.3)	2.0	316	4.3	6.0
	EM15-2	2015	16.1 (12.4 - 19.9)	40.6 (32.7 - 53.5)	3.2	126	5.4	4.2
Theodore	TH15-1	2015	9.9 (7.4 - 12.7)	68.4 (51.6 - 95.7)	1.5		3.3	7.1
Moree	MO15-1	2015	7.4 (4.6 - 11.3)	34.8 (21.4 - 75.5)	1.9	126	2.5	3.6
	MO15-2	2015	6.5 (3.7 - 10)	128.4 (84.5 - 219.6)	1.0	500	2.2	13.4
Darling Downs	DD15-1	2015	7.2 (5.2 - 9.7)	50.2 (35.6 - 78)	1.5	316	2.4	5.2
Namoi	NM15-1	2015	16.3 (8.4 - 31.3)	127.6 (61.7 - 372.1)	1.4	316	5.4	13.3

Locality	Strain	Year	LC_{50} (PPM)	LC_{90} (PPM)	Slope	MEC	RF (LC_{50})	RF (LC_{90})
	NM15-2	2015	4.9 (3.5 - 7.2)	53.6 (29.6 - 129.9)	1.2	126	1.6	5.6
Macintyre	MA15-1	2015	6.8 (4.2 - 14.8)	35.4 (15.9 - 180.9)	1.8	126	2.3	3.7
	MA15-2	2015	3.8 (2.8 - 5.1)	33.2 (21 - 62.8)	1.4	316	1.3	3.5
	MA15-3	2015	4.3 (3.1 - 6)	54.7 (33.9 - 103.4)	1.2	316	1.4	5.7
Emerald	EM16-1	2016	3.2 (1.8 - 5.4)	23 (12.1 - 73.1)	1.5	100	1.1	2.4
Theodore	TH16-1	2016	2.1 (1.4 - 3)	15.8 (10.1 - 30.9)	1.5	100	0.7	1.6
	TH16-2	2016	1.5 (0.7 - 2.6)	33 (16.6 - 103.4)	1.0	100	0.5	3.4
Moree	MO16-1	2016	7.5 (5.5- 10.2)	42.9 (29.3 - 71.5)	1.9	320	2.5	4.5
	MO16-2	2016	9.3 (6.1 - 14.1)	43.7 (26.6 - 95.1)	1.9	320	3.1	4.6
Macintyre	MA16-1	2016	16.6 (11.9 - 22.7)	143.9 (97.7 - 233.9)	1.4	1000	5.5	15.0
	MA16-2	2016	5.2 (3.8 - 7)	31.8 (21.5 - 53.9)	1.6	320	1.7	3.3
	MA16-3	2016	5.6 (3.4 - 8.9)	35.9 (20.5 - 86)	1.6	320	1.9	3.7
Namoi	NM16-1	2016	7.7 (5.9 - 9.8)	24.5 (17.7 - 41.2)	2.6	320	2.6	2.6
	NM16-2	2016	9.4 (6.8 - 12.9)	58.5 (39.2 - 99.5)	1.6	320	3.1	6.1
St George	SG16-1	2016	4 (3 - 5.3)	21.9 (15 - 36.8)	1.7	320	1.3	2.3
	SG16-2	2016	3.2 (2.2 - 4.4)	40.6 (24.1 - 88.1)	1.5	100	1.1	4.2
	SG16-3	2016	3.4 (1.8 - 5.7)	36.3 (18.2 - 123.3)	1.2	100	1.1	3.8
Hillston	HI16-1	2016	4.8 (3.4 - 6.7)	26.74 (17.49 - 48.52)	1.7	1000	1.6	2.8

Appendix 8. Lethal concentration values (LC_{50} & LC_{90}) for foliar clothianidin assays

Locality	Strain	Year	LC_{50} (PPM)	LC_{90} (PPM)	Slope	MEC	RF (LC_{50})	RF (LC_{90})
Lab	Susceptible	2014	8.3 (7.2 - 9.5)	25 (20.3 - 32.8)	2.673			
Emerald	EM14-1	2014	3.6 (1.7 - 5.9)	83.9 (46.5 - 231)	0.94		0.4	3.4
	EM14-2	2014	7.6 (4.2 - 12.5)	44.1 (23 - 204.7)	1.674		0.9	1.8
Theodore	TH14-1	2014	7.9 (3.3 - 14.7)	86.9 (37.4 - 728.3)	1.229	300	1.0	3.5
Moree	MO14-1	2014	18.6 (11.3 - 31.7)	166.4 (80 - 666.6)	1.347		2.2	6.7
	MO14-2	2014	26.8 (16.8 - 45.5)	255.9 (122.1 - 956.8)	1.307		3.2	10.2
St George	SG14-1	2014	21.4 (10.7 - 44.6)	338.3 (122.7 - 3303)	1.069		2.6	13.5
	SG14-2	2014	16.4 (8.8 - 31.6)	139 (61 - 852.1)	1.382		2.0	5.6
	SG14-3	2014	19 (9.3 - 41.5)	195 (74.9 - 1987)	1.267		2.3	7.8
Macintyre	MA14-1	2014	24.7 (16.1 - 41)	149.6 (78 - 497.9)	1.638		3.0	6.0
	MA14-2	2014	30.6 (20.1 - 48.6)	398.6 (194.9 - 1286)	1.149		3.7	15.9
	MA14-3	2014	15 (8.1 - 26.9)	173.1 (77.1 - 879.4)	1.207		1.8	6.9
Namoi	NM14-1	2014	30.9 (20.1 - 50.8)	193.7 (101.9 - 598.1)	1.607		3.7	7.7
	NM14-2	2014	23.8 (15.9 - 37.8)	122.4 (68.1 - 348.6)	1.8		2.9	4.9
Emerald	EM15-1	2015	223.4 (116.9 - 502.6)	20616 (5027 - 306779)	0.652		26.9	825
	EM15-2	2015	82.5 (40.9 - 157.9)	2447 (938.7 - 13465)	0.87		9.9	97.9
St George	SG15-1	2015	117.8 (30.9 - 347)	47036 (8082 - 1965982)	0.4927		14.2	1881
	SG15-2	2015	46.1 (12.5 - 119.3)	19154 (4542 - 268462)	0.4894		5.6	766.2
	SG15-3	2015	1588 (413.1 - 17550)	14462855 (343601 – 8.6E+11)	0.3237		191.3	578514
Namoi	NM15-1	2015	60.5 (12.9 - 178.7)	24852 (4677 - 858695)	0.4903		7.3	994.1
Griffith	GR15-1	2015	4329 (809.8 - 2451892)	56246442 (357394 – 3.4E+19)	0.312		521.6	2249857
Theodore	TH16-1	2016	10.25 (1.5 – 38.1)	3739 (635.1 - 175586)	0.615	1000	1.2	149.6
St George	SG16-2	2016	29 (18.3 - 46.2)	459.4 (243.9 - 1095)	1.068	10000	3.5	18.4
Macintyre	MA16-3	2016	48.03 (26.5 - 85.7)	916.8 (433 - 2713)	1.001	10000	5.8	36.7

Locality	Strain	Year	LC_{50} (PPM)	LC_{90} (PPM)	Slope	MEC	RF (LC_{50})	RF (LC_{90})
Namoi	NM16-2	2016	96.09 (40.7 - 231.5)	3356 (1069 - 23840)	0.83		11.6	134.2
Moree	MO16-1	2016	37.6 (10.2 - 115.2)	2806 (679 - 41926)	0.684		4.5	112.2
Hillston	HI16-1	2016	15.6 (2.2 - 79.3)	1891 (246 - 1955493)	0.5		1.9	75.6

Appendix 9. Lethal concentration values (LC_{50} & LC_{90}) for systemic clothianidin assays

Locality	Strain	Year	LC_{50} (PPM)	LC_{90} (PPM)	Slope	MEC	RF (LC_{50})	RF (LC_{90})
Lab	susceptible	2015	9.3 (6.5 - 13.9)	80.9 (46 - 184.9)	1.365	100		
Emerald	EM15-1	2015	14.9 (11.7 - 19.2)	37.5 (27.6 - 61.3)	3.204	100	1.6	0.5
	EM15-2	2015	15.1 (9.9 - 23)	73 (43.4 - 170.4)	1.868	320	1.6	0.9
Theodore	TH15-1	2015	17.9 (12.1 - 26.2)	136.2 (83 - 274.6)	1.454	560	1.9	1.7
St George	SG15-3	2015	22.2 (14.6 - 34.1)	133.9 (77.7 - 314.3)	1.643		2.4	1.7
	SG15-1	2015	8.4 (6.2 - 11.4)	25.3 (17.6 - 44.9)	2.686	100	0.9	0.3
	SG15-2	2015	7.3 (5.8 - 9.1)	22.4 (16.7 - 34.2)	2.621	100	0.8	0.3
Darling Downs	DD15-1	2015	25.4 (17.4 - 37.8)	165.7 (97.4 - 371.7)	1.574	320	2.7	2.0
Macintyre	MA15-1	2015	25.7 (19.9 - 33.3)	101.8 (73.2 - 159.8)	2.144		2.8	1.3
	MA15-2	2015	30.1 (18.5 - 50.6)	146.1 (79.6 - 443.1)	1.869	320	3.2	1.8
	MA15-3	2015	28.6 (18.6 - 43.8)	116 (70.3 - 275.6)	2.109	320	3.1	1.4
Moree	MO15-1	2015	22.5 (14.9 - 34.3)	82.4 (50.4 - 198.4)	2.271	320	2.4	1.0
	MO15-2	2015	29.2 (19.2 - 46.1)	99.1 (59.3 - 276.6)	2.414	100	3.1	1.2
Namoi	NM15-1	2015	23.1 (18.1 - 29.7)	80.1 (57.7 - 127.6)	2.373	320	2.5	1.0
	NM15-2	2015	26.8 (18.9 - 38.3)	108.1 (69.4 - 216.8)	2.113	320	2.9	1.3
Griffith	GR15-1	2015	15.5 (12.8 - 18.9)	35.9 (28 - 52.2)	3.52	320	1.7	0.4

Appendix 10. Sulfoxaflor lethal concentration values (LC_{50} & LC_{90})

Locality	Strain	Year	LC_{50} (PPM)	LC_{90} (PPM)	Slope	MEC	RF (LC_{50})	RF (LC_{90})
Lab	Susceptible	2014	30.86 (26.61 - 35.87)	74.67 (61.03 - 98.2)	3.339	300		
	susceptible	2015	22.62 (20.19 - 24.97)	36.63 (32.83 - 42.25)	6.122	60		
Emerald	EM14-1	2014	21.98 (16.11 - 29.99)	55.6 (38.62 - 114.9)	3.179	300	0.97	1.52
	EM14-2	2014	21.28 (16.27 - 27.88)	40.69 (30.46 - 74.2)	4.551	100	0.94	1.11
Theodore	TH14-1	2014	19.87 (14.86 - 26.34)	39.22 (28.98 - 74.42)	4.34	100	0.88	1.07
Moree	MO14-1	2014	29.57 (24.74 - 35.2)	82.4 (65.25 - 114)	2.879	300	1.31	2.25
	MO14-2	2014	27.29 (17.17 - 42.06)	88.5 (54.49 - 239.5)	2.508		1.21	2.42
Namoi	NM14-1	2014	25.62 (17.98 - 35.36)	77.46 (52.66 - 155.7)	2.667		1.13	2.11
	NM14-2	2014	32.06 (25.55 - 40.39)	73.14 (55.46 - 113.9)	3.578	300	1.42	2.00
St George	SG14-1	2014	27.38 (23.41 - 32.26)	65.24 (52.56 - 87.89)	3.399	100	1.21	1.78
	SG14-2	2014	20.53 (16.87 - 24.96)	47.51 (36.91 - 71.42)	3.518	300	0.91	1.30
	SG14-3	2014	25.85 (21.78 - 30.53)	73.9 (59.02 - 100.4)	2.809	300	1.14	2.02
Macintyre	MA14-1	2014	87.86 (65.15 - 117.3)	195 (141.4 - 353.1)	3.7		3.88	5.32
	MA14-2	2014	24.66 (19.18 - 31.56)	53.23 (39.78 - 92.73)	3.836	100	1.09	1.45
	MA14-3	2014	26.84 (18.17 - 37.69)	158.6 (108 - 267.4)	2.098	100	1.19	4.33
Ayr	AY09-1R	2014	29.57 (22.1 - 39.56)	158.6 (108 - 267.4)	1.757		1.31	4.33
Emerald	EM15-1	2015	26.46 (22.67 - 30.35)	82.06 (69.73 - 100.3)	2.607	300	1.17	2.24
	EM15-2	2015	33.83 (26.99 - 41.06)	107.6 (85.95 - 144.8)	2.549	500	1.50	2.94

Locality	Strain	Year	LC_{50} (PPM)	LC_{90} (PPM)	Slope	MEC	RF (LC_{50})	RF (LC_{90})
Theodore	TH15-1	2015	24.05 (19.51 - 28.89)	63.34 (50.71 - 86.58)	3.047	100	1.06	1.73
St George	SG15-1	2015	57.8 (51.25 - 64.68)	167.7 (144.7 - 200.6)	2.77	500	2.56	4.58
	SG15-2	2015	33.02 (25.31 - 41.37)	118.6 (90.87 - 170.8)	2.308	500	1.46	3.24
	SG15-3	2015	23.7 (17.22 - 30.48)	112.1 (84.97 - 163)	1.899	500	1.05	3.06
Griffith	GR15-1	2015	36.85 (29.44 - 44.76)	101.9 (80.89 - 140.1)	2.902		1.63	2.78
Moree	MO15-1	2015	45.7 (36.15 - 56.16)	149.8 (116.4 - 211.8)	2.486		2.02	4.09
	MO15-2	2015	46.94 (41.33 - 52.75)	115 (99.43 - 138.1)	3.293	300	2.08	3.14
Namoi	NM15-1	2015	59.4 (50.44 - 69.02)	140.4 (115.6 - 184.5)	3.431	300	2.63	3.83
	NM15-2	2015	68.88 (54.95 - 84.2)	157.9 (123.6 - 234.2)	3.556		3.05	4.31
Macintyre	MA15-1	2015	31.32 (27.48 - 35.31)	79.13 (68.49 - 94.4)	3.184	500	1.38	2.16
	MA15-2	2015	23.99 (18.3 - 30.22)	66.7 (50.82 - 99.96)	2.886	175	1.06	1.82
	MA15-3	2015	15.36 (12.27 - 18.5)	38.32 (30.82 - 52.43)	3.227	100	0.68	1.05

Extension Activities (2013 – 2016)

- Jamie Hopkinson "Silverleaf whitefly update" Northern Farming Systems IPM Researchers Forum. 30-31 July 2013.
- Jamie Hopkinson & Paul Grundy attended St George Grower & Agronomist field day in March 2014 and discussed Silverleaf whitefly management and resistance issues.
- TIMS tech panel meetings 2014, 15 and 16 presented updates on resistance testing
- Research E summary "Safeguarding against Silverleaf whitefly resistance" was presented at the 2014 Australian cotton conference
- Jamie Hopkinson and Richard Sequeira presented "Silverleaf whitefly: Insecticide assumptions & efficacy and the threshold matrix" at the CCA cropping solution seminar at Goondiwindi, 17th July 2014.
- Jamie Hopkinson presented "Aphid taxonomy" at Peracto graduate development program in Toowoomba on the 26th August 2014.
- Jamie Hopkinson attended Toowoomba Ag Show where DAF entomology staff had insect displays including Silverleaf Whitefly. 3rd September 2014
- QDAF entomology participated in the USQ science experience week (24th September 2014). Our involvement included conducting simulated Silverleaf whitefly bioassays.
- Jamie Hopkinson & Paul Grundy attended the CCA cropping solutions seminar in Moree (July 2015) and participated in the "Whitefly Forum". As part of the panel, resistance risk, insecticide usage patterns and impacts on *Eretmocerus* were discussed.
- "Silverleaf whitefly: how to avoid a late season pest problem" was published in December/January 2015 addition of the Australian cottongrower magazine
- Jamie Hopkinson & Paul Grundy "Maintaining control of resistance in SLW" was published in the Winter 2015 edition of Spotlight
- Jamie Hopkinson. "Silverleaf whitefly resistance management" Australian Cotton Research Conference, Toowoomba (September 2015)
- Jamie Hopkinson. "Biology of Minute Two Spotted Ladybird" Australian Cotton Research Conference, Toowoomba (September 2015).

- Jamie Hopkinson. “Developmental biology of the minute two-spotted ladybird beetle, *Diomus notescens*” Australian Entomological Society Scientific Conference, Cairns (September 2015).
- Steph Kramer. “Susceptibility of the silverleaf whitefly parasitoid *Eretmocerus hayati* to insecticides used in cotton” Poster, Australian Cotton Research Conference, Toowoomba (September 2015) and Australian Entomological Society Scientific Conference, Cairns (September 2015).
- Jamie Hopkinson attended “The 3rd Australian Agrochemical Resistance Meeting” 12th November 2015 Melbourne.
- “New insecticides for the control of silverleaf whitefly (SLW) in cotton, and considerations for resistance management” was published at thebeatsheet.com.au January 8th 2016
- Journal article, Hopkinson J.E, Kramer S.M, Zalucki M.P (2016) “Developmental biology and prey preference of *Diomus notescens* Blackburn (Coleoptera: Coccinellidae): A predator of *Aphis gossypii* Glover (Hemiptera: Aphididae)” Biological Control 96 101-107.
- “Ladybirds enjoy a cotton aphid buffet” was published at thebeatsheet.com.au June 6th 2016
- “Keeping SLW’s enemies safe” was published in the Winter 2016 edition of Spotlight
- Steph Kramer “Your ability to manage this problem is under threat” three minute theses. 18th Australian Cotton Conference, August 2nd 2016.

Outcomes

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

For each project milestone we have identified one or more outcomes of significance for the cotton industry.

1.1 Identification of whitefly species/biotype

This study demonstrated that representative samples collected from 14-17 locations per season were all *B. tabaci* MEAM1 (formerly known as B biotype). All samples were 100% identical in sequence, which was expected as this single haplotype has been consistently reported from Australia before.

No native Australian *B. tabaci* or any exotic *B. tabaci* species including any members of the *B. tabaci* Mediterranean species group (formerly known as Q biotype) were identified.

These results provide a comprehensive understanding of the diversity of whitefly present in cotton in Australia and further quantitative evidence that *B. tabaci* MED (Q biotype) is not known to be present in Australia.

2.2 Insecticide resistance of silverleaf whitefly

Results from the project have been used to inform industry of the current status of SLW resistance each year the project has run as part of the Transgenic & Insect Management Strategies review process of the IRMS.

Overall registered products for SLW remain effective and resistance levels remain low. However the detection of resistance to pyriproxyfen at Croppa Creek in northern NSW is a concern and highlights the importance of the IRMS, especially relating to the restricted use of pyriproxyfen.

Resistance to clothianidin is a major concern. Although not registered for SLW; its prevalent use and high toxicity to the whitefly parasitoid (*E. hayati*) means continued high use of this product may lead to widespread SLW management issues.

While extension activities conducted over the course of this project have reinforced the importance of following the IRMS and its recommendation of only one spray per field for pyriproxyfen, it would seem that further action is required to promote this message. The first recorded incidence of pyriproxyfen resistance coupled with background information that would suggest parts of the industry are backsliding with IPM practices is highly significant and is a “red flag” that efforts to encourage better practices need to occur in the 2016/17 season.

3.1 Toxicity of insecticides on *Eretmocerus hayati*

The toxicity of insecticides on the whitefly parasitoid *E. hayati* including sulfoxaflor, cyantraniliprole, fipronil, clothianidin and dinotefuran has been determined. This knowledge has been used to update the cotton pest management guide and provides a ready source of information for growers/agronomists on which products have the best IPM fit when aiming to preserve the biological control service these wasps provide. This information should be emphasised in general IPM extension messages for the coming 2016/17 season as effective biological control is critical for sustainable SLW management.

4.1 Prey consumption studies and 4.2 Prey choice studies

The basic studies undertaken as part of this milestone are the building blocks for potential future studies that should aim to further integrate natural enemy abundance/presence into pest management decisions. Prey choice studies showed that in each case the predators studied preferred cotton aphid over other prey offered (SLW or mealybug). Understanding development and pest suppression capacity of predators allows some simplified modelling to be undertaken that predicts the impact of specific natural enemies.

6. Please describe any:-

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

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

c) required changes to the Intellectual Property register.

Bioassay methodology for insecticide resistance testing was refined. The bioassay for cyantraniliprole was changed to use single leaves rather than seedling plants. This change increased plant survival, which improved the quality of results collected for this insecticide.

Conclusion

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

Based on the outcomes of the project, the take home messages are.

- *Bemisia tabaci* found in our samples was MEAM1 (B biotype) other biotypes were not present in collections.
- One whitefly strain collected from Croppa Creek had resistance to pyriproxyfen (Admiral®). Otherwise SLW remain susceptible to pyriproxyfen.
- Resistance was not found in any of the other registered insecticides (diafenthiuron, cyantraniliprole, spirotetramat, bifenthrin and dinotefuran).
- Widespread resistance to clothianidin (Shield®) was detected in 2014/15 and 2015/16.
- A bioassay of the toxicity of insecticides to *Eretmocerus hayati* found, spirotetramat, flonicamid and cyantraniliprole had low toxicity. Sulfoxaflor and fipronil were moderately toxic. Dinotefuran, clothianidin and bifenthrin were highly toxic.
- The development, prey consumption and prey preference of three predators (*Diomus notescens, Coccinella transversalis* and *Mallada signatus*) of cotton aphid was studied. While each predator developed on cotton aphid; *C. transversalis* and *M. signatus* rates of consumption indicate they are more likely to be effective at pest suppression compared to *D. notescens*.

Extension Opportunities

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

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

Bioassay techniques developed in the project are being used in a new project (DAQ1701) to monitor for insecticide resistance in silverleaf whitefly. Bioassay methods have also been used to collect baseline susceptibility data on an insecticide (Skope®) that is pending registration in cotton for whitefly control.

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

The discovery of resistance to pyriproxyfen during the 2015/16 season was presented at the recent cotton conference. More widespread dissemination of this information will be made via beatsheet blog, cotton grower or spotlight.

(c) for future research.

Bioassay techniques can be applied to look at:

- potentiation (synergism) in a co-formulation e.g. Skope® which contains the active ingredients acetamiprid and emamectin benzoate
- inheritance of resistance to insecticides (bifenthrin and clothianidin)

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

Journal article, Hopkinson J.E, Kramer S.M, Zalucki M.P (2016) "Developmental biology and prey preference of *Diomus notescens* Blackburn (Coleoptera: Coccinellidae): A predator of *Aphis gossypii* Glover (Hemiptera: Aphididae)" Biological Control 96 101-107.

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

None

Part 4 – Final Report Executive Summary

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

Silverleaf whitefly (SLW) collected from cotton at 45 sites over three years were identified to biotype using molecular methods. All 122 individuals were shown to be a single haplotype of *Bemisia tabaci* Middle East Asia Minor 1 (B biotype). No native Australian *B. tabaci* or any exotic *B. tabaci* species including any members of the *B. tabaci* Mediterranean species group (MED - formerly known as Q biotype) were identified. This provides further evidence that *B. tabaci* MED is not present in major cotton production regions of QLD and NSW.

The collected SLW populations were screened for resistance to registered insecticides and new insecticides with registration pending as well as products not registered for whitefly but regularly used on other pests where whitefly may be present. Overall, registered products for SLW remain effective and resistance levels remain low. A single detection of resistance to pyriproxyfen in northern NSW at the end of the 2015/16 season is a concern and highlights the importance of adhering to usage restrictions in the insecticide resistance management strategy (IRMS) and highlights the need to consider IPM practices for all species more broadly.

Resistance has been detected to the widely used neonicotinoid clothianidin. While not registered for SLW control, its prevalent use and high toxicity to the whitefly parasitoid (*E.*

hayati) may increase SLW management issues as this product could be expected to flare SLW abundance.

The toxicity of several insecticides on the whitefly parasitoid *E. hayati* was studied in a laboratory bioassay. Spirotetramat, flonicamid and cyantraniliprole had low toxicity, and sulfoxaflor and fipronil moderate toxicity. Dinotefuran, clothianidin and bifenthrin were all highly toxic. This information has been used to update the Cotton Pest Management Guide natural enemy insecticides impact table. The relative effects of spray decisions on natural enemies should be a point of emphasis for IPM extension messages as the sustainable management of SLW is dependent on having effective biological control.

Development, prey consumption and prey preferences of three predators (minute two-spotted ladybird, transverse ladybird, green lacewing) of cotton aphid were studied. In each case the predators preferred cotton aphid over other prey offered (SLW or mealybug). Additional research is recommended to further develop our understanding of how natural enemies can be integrated into pest management decisions.

Results from the project have been used to inform industry of the current status of SLW resistance as part of the Transgenic & Insect Management Strategies review process of the IRMS. Project extension activities have emphasised the importance of following the IRMS's recommendation of only one spray per field for pyriproxyfen, however further promotion is recommended for this message, as well as IPM practices more generally, particularly regarding the use of thresholds and best practices for all pests, not just SLW.