FINAL REPORT

For Public Release

Part 1 - Summary Details

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Part 3 – Final Report

Background

1. Outline the background to the project.

Bemisia tabaci, commonly known as silverleaf whitefly (SLW) are a major pest of the Australian cotton industry. If present in the crop late in the season while there is open cotton, their honeydew excreta can contaminate lint leading to downgrades due to stickiness and discolouration from sooty mould. Effective management of this pest is therefore critical for the cotton industry to maintain its market positon as a producer of high quality cotton. Silverleaf whitefly have a history of developing resistance to insecticides and a resistance management strategy has been implemented by the cotton industry to reduce the impact of resistance on control options.

This final report details activities of the insecticide resistance monitoring project that monitors the levels of resistance in silverleaf whitefly populations collected from sites across each of the major cotton production valleys.

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. Whitefly species identification

This objective was met in the first year of the project. Subsamples from each population collected were identified to species using molecular diagnostics.

More screening was completed between 17/18 and 18/19 with results reported in the subsequent project (DAQ2001 '*Sustainable SLW management through improved insect resistance monitoring*'). This milestone supports biosecurity surveillance by identifying which cryptic species are present in populations collected from cotton. Other invasive members of the *Bemisia tabaci* species complex pose a significant biosecurity risk to Australian agriculture so detection is critical.

2. Resistance monitoring of silverleaf whitefly

This milestone was fully met by testing for the presence of insecticide resistance in SLW populations each year of the project. Populations of SLW were collected from widespread geographic areas representing each of the major cotton production regions in eastern Australia. Results of resistance testing were presented annually at the transgenic and insect management strategies (TIMS) insecticide technical panel meetings, and the insecticide resistance management strategy (IRMS) for whitefly modified to reflect changes in SLW resistance levels.

Resistance to spirotetramat was detected in Australia in the early stages of this project; additional research in collaboration with Bayer was completed over the duration of the project.

3. Impact of new insecticides on the whitefly parasitoid *Eretmocerus hayati*

This milestone was completed to a limited extent. The insecticides tested included bifenthrin, pyriproxyfen, diafenthiuron, acetamiprid, emamectin benzoate and afidopyropen. A residue experiment examined afidopyropen, acetamiprid and emamectin benzoate, but only 2 time intervals out of 5 were completed due to problems with wasp viability.

4. Extension and reporting

This milestone was fully met. Results from this project were communicated to industry using a variety of extension techniques including presentations at Crop Consultants Australia (CCA) seminars, CottonInfo extension events (agronomist/grower meetings, webinars, YouTube videos) and industry media (Spotlight, Australian cottongrower).

Methods

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

Methods used in this project have been developed in previous projects (DAQ1403) and have been sourced from the Insecticide Resistance Action Committee (IRAC methods), journal articles (see references) and collaborators.

Whitefly species identification

As the *Bemisia tabaci* species complex is morphologically indistinguishable, molecular diagnostics are used to separate the species. Approximately 200 adults from each whitefly population collected by the project were preserved in 90% ethanol and stored at -20°C until used. For each population, genomic DNA of adult whiteflies was isolated using a silica spin-column method. Whitefly identification was based on the 3' barcoding region of the mitochondrial cytochrome oxidase 1 (mtCOI) gene. This region was amplified and sequenced using primers C1-J-2195 (5'-TTGATTTTTTGGTCATCCAGAAGT-3') and TL2-N-3014 (5'-TCCAATGCACTAATCTGCCATATTA-3'). Sanger sequencing of amplicons was performed by Macrogen (Seoul, South Korea). Sequence analysis was performed using Geneious version 9.1.7, with sequence-based comparisons performed using a global reference dataset of whitefly haplotype partial mtCOI sequences downloaded from GenBank.

Additional testing of populations was conducted by University of Canberra (UC) honours student, Cao Fang, using Sanger sequencing and next-generation sequencing (NGS) (Fang 2019).

Resistance monitoring of silverleaf whitefly

Insecticides tested

A total of 10 insecticides were tested over the duration of the project (Table 1). For significant insecticides (e.g. pyriproxyfen) and new insecticides where baseline data is required, each population collected was tested, while products of lesser significance e.g. bifenthrin, only one population per region was tested.

Collection of insects and culturing

Whitefly were collected from commercial cotton crops by the project team or sent to the lab by agronomists who collected on our behalf. A list of where populations were collected is included in Appendix 1. Whitefly adults were collected using blower vacs with a gauze collection sleeve placed over the entrance of the machine. Collected whitefly were transferred into field cages with plant material. In the lab whiteflies were transferred from the field cage into colony cages to establish glasshouse populations. Some populations were started from nymph infested leaves sent in by agronomists. Leaves were placed into a cage and as adult whiteflies emerged they were aspirated into a colony cage to separate them from other insects i.e parasitoids in the sample.

Populations were maintained as discrete generations. The next generation was established on a new plant placed inside the colony cage by allowing 3 to 4 days of egg lay by the parental generation. After the egg laying period the adults were removed and the old plant discarded.

A susceptible population (SU07-1) was cultured over the duration of the project. Four populations each carrying resistance were also kept in culture for the duration of the project. These included PB1510R (bifenthrin resistant), AY09-1R (pyriproxyfen resistant), GR15-1R (neonicotinoid resistant) and AY16-1R (spirotetramat resistant). Each resistant population was 'pressured' every generation with a dose of insecticide that selects for resistant individuals. Cotton plants, Sicot 714 B3F without insecticide treatment were germinated in control temperature rooms and then grown in large insect proof cages inside a glasshouse. Cotton plants were used for all bioassays and as a host for maintaining populations.

Bioassays

Bioassays tested 5 to 7 concentrations of each insecticide, plus a control, with each dose replicated 5 times. Four types of assays were used to test insecticides including:

- Egg bioassay – used to test pyriproxyfen. Leaves with freshly laid eggs are treated and assessed at 10 days by counting number of unhatched eggs (Horowitz et al., 2002).
- Egg/nymph bioassay – used to test cyantraniliprole. Leaves with freshly laid eggs are placed into vials containing the active ingredient which is taken up by the plant. Nymph mortality is assessed at 13 days (Li et al., 2012).
- Nymph assays – used to test spirotetramat and buprofezin. Leaves with nymphs at the 2nd instar are dipped in the active ingredient and mortality of nymphs is assessed 11 days later (Nauen et al., 2008).
- Adult assay – used for all other products. Leaves are dipped in insecticide, left to dry, then adults are caged onto the treated leaves using clip cages. Mortality assessments are made at 2, 3, or 4 days depending on the product tested (Castle and Prabhaker 2013).

Populations with suspected resistance were 'pressured' i.e. tested further by exposing them to whole plants sprayed with a higher concentration of insecticide and recording survival several day later depending on the insecticide tested.

Molecular screening for resistance alleles

As part of Cao Fang's honours thesis at UC, screening of populations for a known pyrethroid resistance allele was completed using NGS (Fang 2019).

Data analysis

Non-pooled bioassay data were analysed by probit (Genstat 19th edition). From this analysis lethal concentrations (LC_{50} – $LC_{99.9}$) and their 95% fiducical limits were calculated. For each assay the lowest dose that killed 100% of tested insects; known as the minimum effective concentration was recorded. For each population, a resistance ratio (RR) was derived by dividing the calculated LC50 of the field collected populations by the value of a reference susceptible population (SU 07-1).

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

Insecticide	Trade name	Conc.	Dose range (mg/L a.i.)	Bioassay type	Development stage targeted	Duration (days)
Pyriproxyfen	Admiral®	100 g/L	0.001 - 10	leaf dip	eggs	10
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
Buprofezin	Applaud®	440 g/L	0.3 - 100	leaf dip	2nd instar nymphs	11
Pyriproxyfen	Admiral®	100 g/L	0.001 - 10	leaf dip	eggs	10
Afidopyropen	Versys®	100 g/L	0.3 - 300	leaf dip	adults	4
Diafenthiuron	Pegasus®	500 g/L	3 - 300	leaf dip	adults	3
Acetamiprid	Intruder®	225 g/L	0.3 - 300	leaf dip	adults	3
Emamectin benzoate	Affirm®	17 g/L	0.03 - 30	leaf dip	adults	3
Dinotefuran	Starkle®	200 g/kg	0.3 - 320	leaf dip	adults	3
Bifenthrin	Talstar®	250 g/L	1 - 1000	leaf dip	adults	2

After the detection of spirotetramat resistance in whitefly collected out of the horticultural regions of Ayr and Bowen, reciprocal crossing, resistance stability and sterility experiments were conducted.

- Resistance stability experiments consisted of repeat bioassays of two populations (AY16-1 and BO16-1) every generation for 12 months in the absence of further resistance selection. Each bioassay comprised two doses (100 and 300 mg/L) and a control replicated 5 times. Stability was determined by comparing mortality recorded at each generation.
- The reciprocal crossing experiments involved crossing a spirotetramat resistance population (AY16-1R) with a susceptible population (SU07-1) and assaying the offspring of these crosses to spirotetramat doses ranging from 1–1000 mg/L. Survivorship from these bioassays was then used to determine degree of dominance at each dose (Crowder et al., 2008).
- To assess if spirotetramat resistance affected the insecticide's ability to induce sterility, adult fecundity (eggs laid) and fertility (viable nymphs) was assessed for a susceptible (SU07-1) and resistant population (AY16-1R) at doses ranging from 8 to 1000 mg/L (Nauen et al., 2008).

***Impact of new insecticides on the whitefly parasitoid* Eretmocerus hayati**

The toxicity of recently registered insecticides for SLW control was determined using a combination of direct and residual exposure (Table 2). Pyriproxyfen was included as a 'low toxicity' standard and bifenthrin was included as a 'very high toxicity' standard. Diafenthiuron was included as previous studies had given inconclusive results. Direct exposure was conducted using a potter spray tower. The potter tower was run with an air compressor calibrated to deliver a spray volume of 100 L/ha. Cotton leaves were sprayed on the abaxial surface and left for 30 minutes to dry, their petiole was trimmed with a scalpel and placed into a vial of water. A clip cage was placed onto the leaf and 15–20 wasps were added to each cage (experimental unit). In place of the cork stopper used in SLW bioassays a small ball of cotton wool wrapped in perforated parafilm was used to seal the cage opening. Using an eye dropper, honey water solution (10% honey) was used to moisten the cotton wool, which provided the wasp a source of nutrition for the duration of the bioassay. Mortality was assessed at 24, 48 and 72 h after exposure using a stereo microscope.

To evaluate the residual toxicity of acetamiprid, emamectin benzoate and afidopyropen, cotton plants were sprayed with a mini boom sprayer at label rates (including additive, Table 2) and leaves with dried residue were collected at 6 and 9 days after application. Using the same procedure as the direct spray, *E. hayati* were exposed to residues for 48 h. Mortality was assessed at 24 and 48 h using a stereo microscope.

For both experiments, mortality data was arcsine transformed and analysed using a one-way ANOVA to compare means at each time interval.

Table 2. Application rates for direct and residual toxicity experiments

Experiment	Insecticide	Application rate (g ai/ha)	Additive
direct	bifenthrin	80	
direct	pyriproxyfen	50	
direct & residue	emamectin benzoate	10	1% hasten
direct & residue	acetamiprid	68	1% hasten
direct & residue	afidopyropen	35	0.2% hasten
direct	diafenthiruon	300	

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Results

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

Whitefly species identification

The *B. tabaci* species complex present in the field collected populations was identified using molecular diagnostics. In 2016-2017, the dominant whitefly was *B. tabaci* Middle East-Asia Minor 1 (MEAM1), with *B. tabaci* Australia (AUSI) found at only one site near Goondiwindi, where it represented 7.9% of the population.

Resistance monitoring of silverleaf whitefly

a) Pyriproxyfen

At the end of the 2016-17 season, the 19 populations collected from cotton-growing regions were tested by bioassay to determine levels of resistance to pyriproxyfen, including two populations collected off weeds in the Gwydir valley during the winter months. Based on the survival at a 'pressuring' dose of 32 mg/L pyriproxyfen and bioassay data, 11 populations were resistant (Table 3). While MA17-3 had a high resistance factor, 100% mortality was achieved at the discriminating dose of 10 mg/L and no viable offspring were produced when adults were exposed to a plant treated with 32 mg/L of pyriproxyfen. Both populations collected from weeds survived when treated with 32 mg/L of pyriproxyfen.

Towards the end of the 2017-18 cotton season, 16 populations of whitefly were collected and screened for resistance to pyriproxyfen. Based on bioassay data and survival on plants sprayed with 32 mg/L of pyriproxyfen, eight populations were resistant (Table 3). This season included a whitefly population collected from the Macquarie valley that was susceptible to pyriproxyfen.

Table 3. Pyriproxyfen bioassay summary data 2016-17 and 2017-18

Season	Population	LC50 (mg/L)	Minimum Effective Conc. (mg/L a.i.)	Mortality (%) at Discriminating Dose (10 mg/L a.i.)	Resistance Ratio (LC50)	Resistant
2016-18	Susceptible (SU07-1)	0.02	0.1	100	-	-
2016-17	EM17-1	0.01	1	100	0.6	-
	TH17-1	0.001	0.1	100	0.1	-
	TH17-2	0.001	0.01	100	0.1	-
	DD17-1	0.01	10	100	0.7	-
	SG17-1	0.27	-	98	15	+
	SG17-2	0.17	10	100	9	-
	SG17-3	0.29	-	99	16	+
	MA17-1	0.10	10	100	5	-
	MA17-2	0.30	-	98.7	16	+
	MA17-3	0.46	10	100	25	-
	MO17-1	0.49	-	94.2	26	+
	MO17-2	2.10	-	83.7	113	+
	MO17-3	0.29	10	100	16	+
	MO17-4	0.71	-	99	38	+
	MO17-5*	0.25	10	100	13	+
	MO17-6*	0.77	-	96	41	+
	NM17-1	0.28	-	98	15	+
	NM17-2	1.2	-	89.3	64	+
	HI17-1	0.04	10	100	2	-
2017-18	EM18-1	0.01	1	100	0.5	-
	TH18-1	0.01	1	100	0.4	-
	SG18-1	0.69	-	96	37.3	+
	SG18-2	0.22	-	98.7	11.7	+
	SG18-3	0.17	10	100	9.0	+
	MA18-1	0.08	-	99.3	4.1	+
	MA18-2	0.17	10	100	8.9	+
	MA18-3	0.13	10	100	7.0	-
	MO18-1	0.27	-	99.3	14.3	+
	MO18-2	0.53	10	100	28.4	+
	MO18-3	0.33	-	98	18.0	+
	NM18-1	0.04	10	100	2.0	-

Season	Population	LC50 (mg/L)	Minimum Effective Conc. (mg/L a.i.)	Mortality (%) at Discriminating Dose (10 mg/L a.i.)	Resistance Ratio (LC50)	Resistant
	NM18-2	0.03	10	100	1.7	-
	NM18-3	0.09	10	100	4.6	-
	WA18-1	0.08	10	100	4.6	-
	GR18-1	0.03	1	100	1.7	-

*collected from weeds during winter 2017

Nineteen populations were tested in the 2018-19 season, of those 6 populations had survivors at the discriminating dose of 3 mg/L (Table 4). The discriminating dose was reduced from 10 to 3 mg/L based on the 'pressuring' results of the previous season which were indicative of false negatives when using 10 mg/L.

With further testing, including both assaying using higher concentrations (10 and 30 mg/L) and testing on sprayed plants (30, 100 & 320 mg/L) differences in level of resistance could be determined. NM19-1, NM19-3, MO19-2 and MO19-3 all died in the lab assay at 10 mg/L and for MU19-1 all eggs were dead at 30 mg/L, while 1.3% of the NM19-2 population assayed survived 30 ppm (2 out of 150).

Populations MU19-1, MO19-2, MO19-3, NM19-1, NM19-2, and NM19-3 were selected for resistance with increasing doses of pyriproxyfen by spraying whole plants. All produced offspring on plants treated with 30 mg/L, but only populations NM19-1 and NM19-2 survived doses above 100 mg/L

Table 4. Pyriproxyfen bioassay summary data 2018-19

Population	LC50 (mg/L)	Minimum Effective Conc. (mg/L a.i.)	Mortality (%) at Discriminating Dose (3 mg/L a.i.)	Resistance Ratio (LC50)	Resistant
Susceptible (SU07-1)	0.02	0.1	100	-	-
EM19-1	0.02	3	100	0.9	-
EM19-2	0.01	1	100	0.4	-
TH19-1	0.02	1	100	0.9	-
DD19-1	0.02	1	100	1.1	-
SG19-1	0.10	3	100	5.5	-
SG19-2	0.27	3	100	14.3	-
SG19-3	0.10	3	100	5.2	-
MU19-1	0.25	-	92	13.3	+
MA19-1	0.06	3	100	3.4	-
MA19-2	0.10	3	100	5.3	-
MA19-3	0.13	3	100	7.2	-
MO19-1	0.14	3	100	7.4	-
MO19-2	0.30	-	89.9	16.2	+
MO19-3	0.46	-	91.3	24.7	+
NM19-1	0.13	-	98	7.3	+
NM19-2	0.18	-	93	9.9	+
NM19-3	0.19	-	94.7	10.4	+
WA19-1	0.10	3	100	5.5	-
GR19-1	0.06	3	100	3.3	-

b) Diafenthiuron

Fifty whitefly populations were tested for resistance to diafenthiuron over the duration of the project (Table 5). All populations were susceptible, with 100% mortality reached at a doses below or equal to the discriminating dose of 300 mg/L.

Table 5. Diafenthiuron bioassay summary data 2016-17, 2017-18 and 2018-19

Season	Population	LC50 (mg/L a.i.)	Minimum Effective Conc. (mg/L a.i.)	Resistance Ratio (LC50)
2016-19	Susceptible (SU07-1)	33.4	300	-
2016-17	EM17-1	13.3	100	0.4
	TH17-1	27.5	300	0.8
	TH17-2	8.9	30	0.3
	DD17-1	10.2	100	0.3
	SG17-1	19.9	100	0.6
	SG17-2	20.6	100	0.6
	SG17-3	15.5	30	0.5
	MA17-1	27.8	300	0.8
	MA17-2	10.2	100	0.3
	MA17-3	15.4	100	0.5
	MO17-1	9.5	100	0.3
	MO17-2	12.0	100	0.4
	NM17-1	8.1	30	0.2
	NM17-2	7.0	100	0.2
	HI17-1	8.0	30	0.2
2017-18	EM18-1	22.2	100	0.7
	TH18-1	7.4	100	0.2
	SG18-1	40.6	300	1.2
	SG18-2	29.4	100	0.9
	SG18-3	41.7	300	1.2
	MA18-1	29.4	300	0.9
	MA18-2	16.7	100	0.5
	MA18-3	15.2	100	0.5
	MO18-1	23.8	100	0.7
	MO18-2	21.94	100	0.7
	MO18-3	13.1	100	0.4
	NM18-1	16.9	300	0.5
	NM18-2	10.2	100	0.3
	NM18-3	27.3	300	0.8
	WA18-1	14.7	100	0.4
	GR18-1	11.7	100	0.3
2018-19	EM19-1	22.7	300	0.7
	EM19-2	25.9	100	0.8
	TH19-1	14.1	100	0.4
	DD19-1	16.9	300	0.5
	SG19-1	16.8	100	0.5
	SG19-2	13.5	100	0.4
	SG19-3	27.2	100	0.8
	MU19-1	7.7	30	0.2
	MA19-1	19.9	100	0.6
	MA19-2	27.1	300	0.8
	MA19-3	25.5	300	0.8
	MO19-1	41.1	300	1.2
	MO19-2	22.6	300	0.7
	MO19-3	22.8	300	0.7
	NM19-1	26.2	100	0.8
	NM19-2	22.5	100	0.7
	NM19-3	23.0	100	0.7
	WA19-1	38.0	300	1.1
	GR19-1	10.2	300	0.3

c) Bifenthrin

Resistance testing was completed on 27 populations over the duration of the project (Table 6). With a discriminating dose of 300 mg/L the following populations were resistant;

- **2016-17:** MA17-1
- **2017-18:** EM18-1, MA18-1, MA18-3, NM18-1, WA18-1 & GR18-1.
- **2018-19:** SG19-3, MU19-1, NM19-3.

Screening for resistance based on presence of *vgsc* gene (L9251), found that all sites from Goondiwindi – Macintyre (MA) in 2016-17 had resistance, with 1 to 4% of the population carrying the mutation. Other sites with resistance included MO17-1 (4%) and TH17-1 (1.3%).

Table 6. Bioassay summary data for bifenthrin 2016-19.

Season	Population	LC50 (mg/L a.i.)	Minimum Effective Conc. (mg/L a.i.)	Resistance Ratio (LC50)
2016-19	Susceptible (SUo7-1)	2.5	32	-
2016-17	EM17-1	4.8	100	1.9
	TH17-1	3.8	100	1.5
	DD17-1	3.9	100	1.6
	SG17-2	4.9	100	2.0
	MA17-1	5.3	1000	2.1
	MO17-2	4.3	100	1.7
	NM17-1	4.0	100	1.6
	HI17-1	6.6	320	2.6
2017-18	EM18-1	7.8	1000	3.2
	TH18-1	1.8	100	0.7
	SG18-1	1.9	320	0.8
	MA18-1	12.0	1000	3.3
	MA18-3	15.0	-	3.2
	MO18-1	5.6	320	1.5
	NM18-1	17.3	-	4.9
	WA18-1	5.6	1000	1.7
	GR18-1	8.9	1000	2.6
2018-19	EM19-1	3.5	320	1.4
	TH19-1	1.7	100	0.7
	DD19-1	3.4	320	1.4
	SG19-3	4.6	1000	1.9
	MU19-1	3.7	1000	1.5
	MA19-2	2.8	100	1.1
	MO19-2	6.8	100	2.7
	NM19-3	3.3	1000	1.3
	WA19-1	6.3	320	2.6
	GR19-1	8.5	320	3.5

d) Spirotetramat

Resistance to spirotetramat was detected in two populations collected from horticulture in Bowen and Ayr during 2016 (Figure 1).

Testing of 35 populations collected from cotton production valleys found that with the exception of one population from Emerald in 2019 (EM19-2), populations were susceptible (Table 7). Resistance detected in EM19-2 was low with 3% (4 alive out of 150 tested) of the population alive at a discriminating dose of 100 mg/L. The resistance ratio of this population was 1.5 fold.

Table 7. Bioassay summary data for spirotetramat 2016-19.

Season	Population	LC50 (mg/L a.i.)	Minimum Effective Conc. (mg/L a.i.)	Resistance Ratio (LC50)
2016-19	Susceptible (SU07-1)	4.1	100	-
2016-17	EM17-1	7.8	300	1.9
	TH17-1	17.6	100	4.3
	TH17-2	10.7	100	2.6
	DD17-1	7.8	300	1.9
	SG17-2	6.1	300	1.5
	MA17-1	4.7	100	1.1
	MO17-2	7.7	100	1.3
	NM17-1	5.3	100	1.3
	HI17-1	6.2	100	1.5
2017-18	EM18-1	4.0	100	1.0
	TH18-1	5.3	100	1.3
	SG18-1	4.4	100	1.1
	MA18-3	2.0	10	0.5
	MO18-1	5.4	10	1.3
	NM18-1	5.9	100	1.5
	WA18-1	7.0	10	1.7
	GR18-1	7.5	100	1.8
2018-19	EM19-1	3.0	30	0.7
	EM19-2	6.1	-	1.5
	TH19-1	4.3	100	1.0
	DD19-1	2.9	100	0.7
	SG19-1	4.8	100	1.2
	SG19-2	8.5	100	2.1
	SG19-3	2.4	100	0.6
	MU19-1	3.5	30	0.9
	MA19-1	2.9	100	0.7
	MA19-2	2.5	100	0.6
	MA19-3	6.6	100	1.6
	MO19-1	3.7	30	0.7
	MO19-2	6.0	100	1.5
	MO19-3	4.8	100	1.2
	NM19-1	11.9	100	2.9
	NM19-2	6.2	30	1.5
	NM19-3	5.5	30	1.4
	WA19-1	4.7	30	1.2
	GR19-1	3.4	100	0.8
	EM19-2P	41.2	-	10.1
	EM19-2SURV	68.3	-	16.7

To further test EM19-2, two actions were taken. The first was to 'pressure' the EM19-2 population with a dose of 1000 mg/L that would select for resistance, resulting in EM19-2P. The second was to keep survivors of the bioassay and establish a glasshouse population EM19-2SURV. The second generation of the two populations were assayed with spirotetramat up to 1000 mg/L and both populations had survivors at that dose confirming the original population contained resistant individuals (Figure 2).

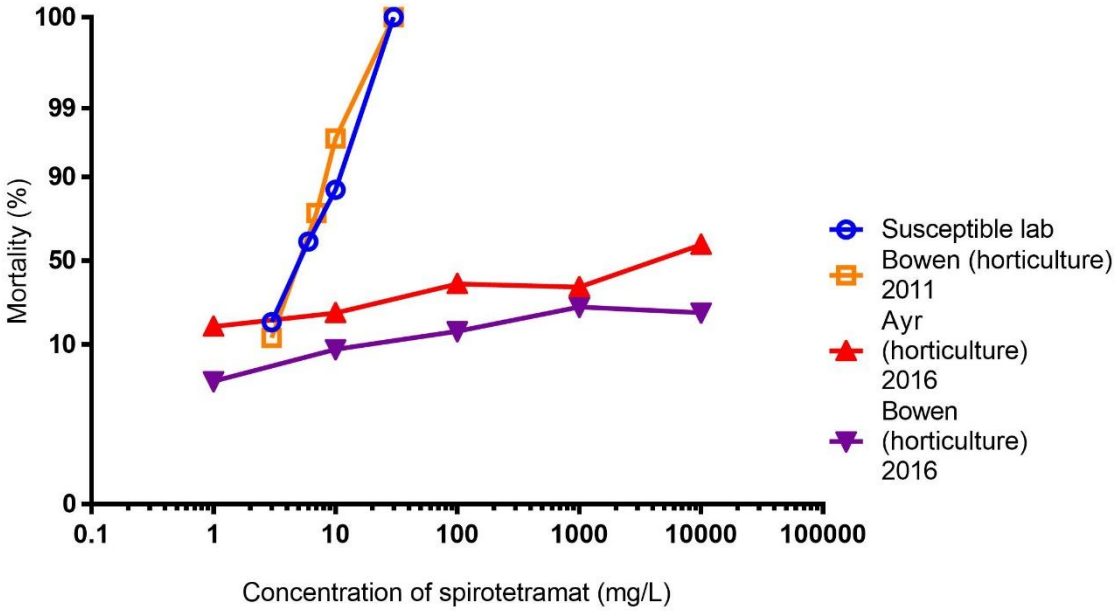

Figure 1. Dose response of *Bemisia tabaci* populations collected from Bowen (2011, 2016), Ayr (2016) and a lab strain susceptible to spirotetramat.

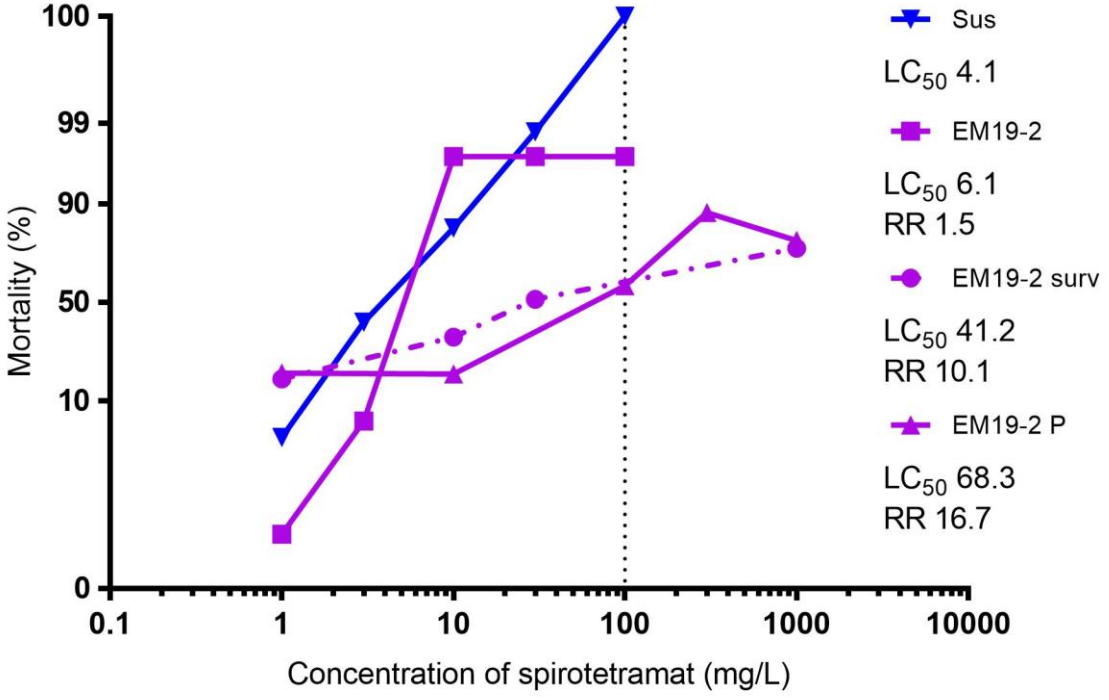

Figure 2. Dose response of *Bemisia tabaci* populations, lab susceptible (Sus) and Emerald (EM 19-2). The response of a population derived from survivors of the initial bioassay (EM19-2 surv) and population that survived 'pressuring' at 1000 mg/L (EM19-2 P) was tested by bioassay.

e) Resistance stability, inheritance and sterility experiments

As part of a collaborative study with Bayer we have examined the stability of resistance and investigated the inheritance of resistance.

In the absence of further selection pressure, populations from Ayr (AY16-1) and Bowen (BO16-1) remained resistant over 12 generations. While there was variability in mortality between generations, the two population never reached 100% mortality at either of the diagnostic doses (100 & 300 mg/L). Average mortality at 100 mg/L was 37.1% for AY16-1 and 30.6% for BO16-1.

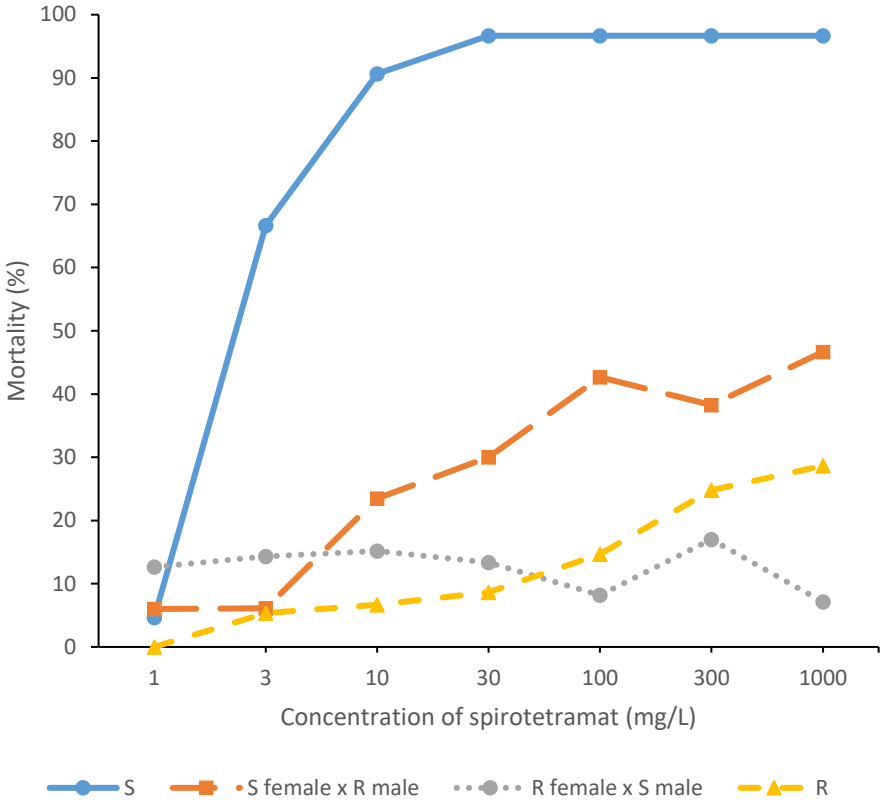

Figure 3. Mortality caused by spirotetramat (±SE) for four bioassays. Susceptible strain SU07-1 (S), resistant strain AY16-1 (R), hybrid nymphs were progeny of (S♀ X R♂) and (R♀ X S♂).

A reciprocal crossing experiment of two populations; one susceptible (SU07-1) and the other resistant to spirotetramat (AY16-1R) was completed to determine mode of inheritance. The progeny of these crosses were bioassayed with spirotetramat with doses ranging from 1 to 1000 mg/L (Figure 3). For the two crosses, dominance was calculated at 0.8 (S♀ X R♂) and 0.95 (R♀ X S♂) (Figure 4) i.e. dominant inheritance.

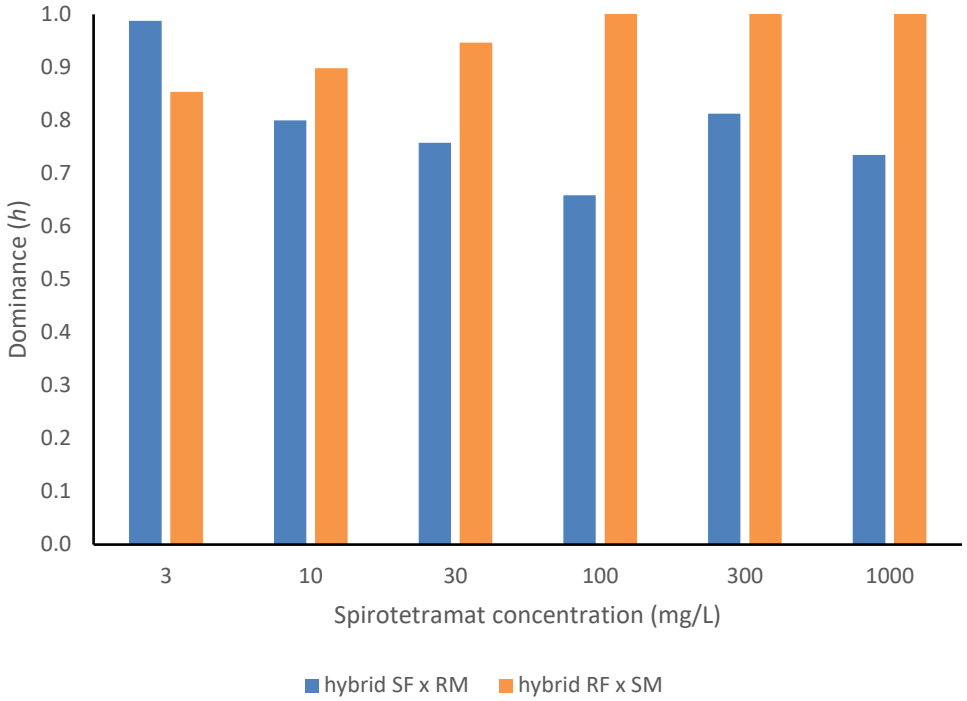

Figure 4. Dominance of resistance (h) in a spirotetramat SLW nymph bioassay. Nymphs were hybrid progeny of susceptible (SU07-1) and resistant (AY16-1R) parents, ♀SU07 -1 (SF) x ♂ AY16-1R (RM) and ♀AY16-1R (RF) x ♂SU07-1 (SM)

The sterility experiments found that neither fecundity nor fertility of the resistant population (AY16-1R) was affected when treated with spirotetramat, compared to the susceptible population (SU07-1) where sterility was induced (Figure 5).

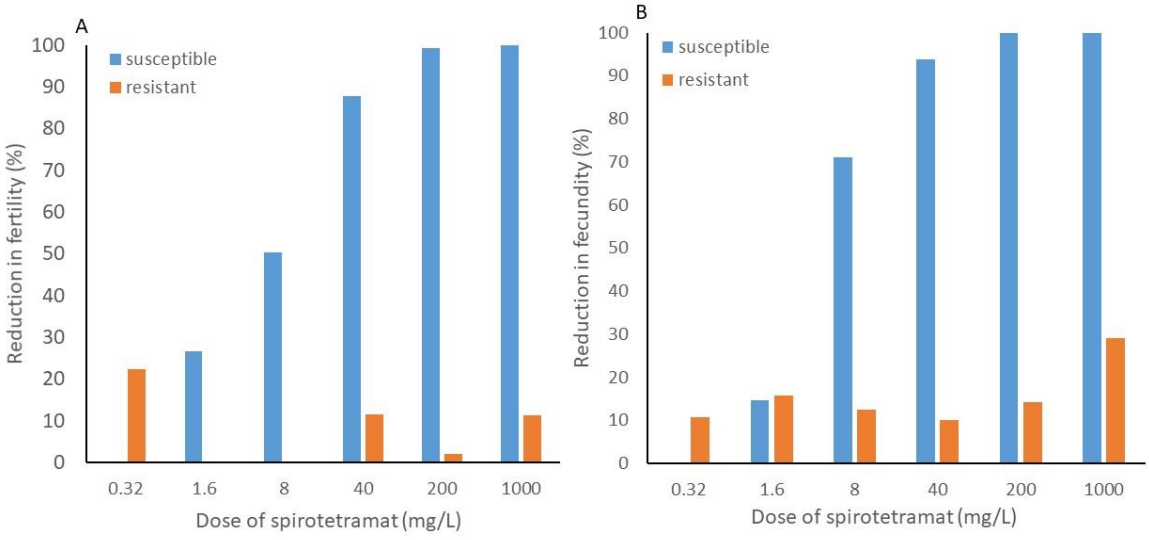

Figure 5. Reduction in fertility (A) and fecundity (B) after exposure to spirotetramat in susceptible (SU07-1) and resistant (AY16-1R) strains.

f) Dinotefuran

In total 33 assays were completed testing whitefly for resistance to dinotefuran (Table 8). Only one population, DD17-1 had a survivor at the discriminating dose of 320 mg/L. When this population was selected for resistance with a dose of 1000 mg/L there were no survivors. At this time there is no evidence of resistance to dinotefuran.

Table 8. Bioassay summary data for dinotefuran 2016-19

Season	Population	LC50 (mg/L a.i.)	Minimum Effective Conc. (mg/L a.i.)	Resistance Ratio (LC50)
2016-17	Susceptible (SU07-1)	12.3	85	-
2016-17	EM17-1	20.8	100	1.7
	TH17-1	9.7	100	0.8
	TH17-2	10.1	100	0.8
	DD17-1	30.3	-	2.5
	SG17-1	10.9	100	1.7
	SG17-2	13.8	100	1.1
	SG17-3	7.1	100	0.6
	MA17-1	15.3	100	1.2
	MA17-2	8.7	32	0.7
	MA17-3	5.5	100	0.4
	MO17-1	20.2	320	1.6
	MO17-2	13.2	100	1.1
	NM17-1	9.0	100	0.7
	MN17-2	10.5	320	0.9
	HI17-1	11	320	0.9
2017-18	EM18-1	7.5	100	0.6
	TH18-1	8.3	100	0.7
	SG18-1	10.4	100	0.8
	MA18-2	6.6	100	0.5
	MO18-1	5.8	100	0.5
	NM18-1	3.6	32	0.3
	WA18-1	22.4	100	1.8
	GR18-1	13.5	100	1.1
2018-19	EM19-1	11.8	160	1.0
	TH19-1	4.6	50	0.4
	DD19-1	13.8	160	1.1
	SG19-1	8.6	100	0.7
	MU19-1	9.2	100	0.8
	MA19-1	17.8	320	1.4
	MO19-3	16.2	320	1.3
	NM19-1	6.8	100	0.6
	WA19-1	5.6	100	0.5
	GR19-1	18.0	320	1.4

g) Acetamiprid

Acetampirid is one of the active ingredients of the co-formulation Skope, the other being Emamectin benzoate. The two actives were tested separately. In total, 49 populations were tested over the 3 years of the project (Table 9).

Table 9. Bioassay summary data for acetamiprid 2016-17, 2017-18 and 2018-19

Season	Population	LC50 (mg/L a.i.)	Minimum Effective Conc. (mg/L a.i.)	Resistance Ratio (LC50)
2016-19	Susceptible (SU07-1)	2.7	300	-
2016-17	EM17-1	5.9	100	2.2
	TH17-1	2.2	30	0.8
	TH17-2	3.8	30	1.4
	DD17-1	4.7	100	1.8
	SG17-1	11.4	100	4.2
	SG17-2	6.5	100	2.4
	SG17-3	10.9	300	4.0
	MA17-1	14.2	300	5.2
	MA17-2	11.0	300	4.1
	MA17-3	17.6	-	6.5
	MO17-1	5.1	30	1.9
	MO17-2	1.5	30	0.6
	NM17-1	11.9	300	4.4
	NM17-2	7.6	300	2.8
	HI17-1	21.3	300	7.9
2017-18	EM18-1	14.5	300	5.4
	TH18-1	7.4	100	2.7
	SG18-1	9.3	100	3.4
	SG18-2	6.3	100	2.3
	SG18-3	6.3	100	2.3
	MA18-1	9.7	100	3.6
	MA18-2	13.5	100	5.0
	MA18-3	8.0	100	3.0
	MO18-1	25.6	100	9.4
	MO18-2	12.1	100	4.5
	MO18-3	9.4	100	3.5
	NM18-1	10.2	100	3.8
	NM18-2	13.1	100	4.8
	NM18-3	13.9	100	5.1
	WA18-1	5.0	100	1.9
	GR18-1	3.6	100	1.3
2018-19	EM19-1	4.3	30	1.6
	EM19-2	12.9	300	4.4
	TH19-1	5.1	100	1.9
	DD19-1	15.5	300	5.7
	SG19-1	8.9	300	3.3
	SG19-2	10.7	100	3.9
	SG19-3	9.6	300	3.6
	MU19-1	4.8	100	1.8
	MA19-1	32.2	300	11.9
	MA19-2	13.4	300	5.0
	MA19-3	21.5	-	7.9
	MO19-1	10.3	100	3.8
	MO19-2	9.8	100	3.6
	MO19-3	14.1	300	5.2
	NM19-1	2.9	30	1.1
	NM19-2	3.8	300	1.4
	NM19-3	2.1	30	0.8
	WA19-1	6.4	100	2.4
	GR19-1	5.6	100	2.1

In 2016-17 two populations with high resistance ratios (MA17-3 and HI17-1) were selected for resistance in the glasshouse at 1000 mg/L, but neither population had surviving whitefly at 72 h post treatment, indicating resistance individuals were not present in these populations. During 2017-18 populations were susceptible with all 16 populations effectively controlled at or below 300 mg/L.

In 2018-19 resistance, a population from Macintyre valley (MA19-3) had survivors at 300 mg/L which was indicative of a *Prima Facie* detection of resistance (Figure 6). This populations was selected for resistance in the glasshouse at a dose of 1000 mg/L which resulted in a small number of survivors, again an indication of resistance. The population was then left unselected i.e. no 'pressuring' for one generation to allow recovery to sufficient numbers for testing. However when retested at a dosage range of 3–1000 mg/L this population (MA19-3P), and was effectively controlled at 300 mg/L with an LC50 of 18.1 (14.3–22.6) mg/L. A further selection was completed at 2000 mg/L and resulted in no survivors. The bioassay results and the lack of survivors from the next round of 'pressuring indicate a lack of resistance in the population. All other populations tested from the 2018-19 season were controlled at doses equal to or below 300 mg/L indicating they are susceptible.

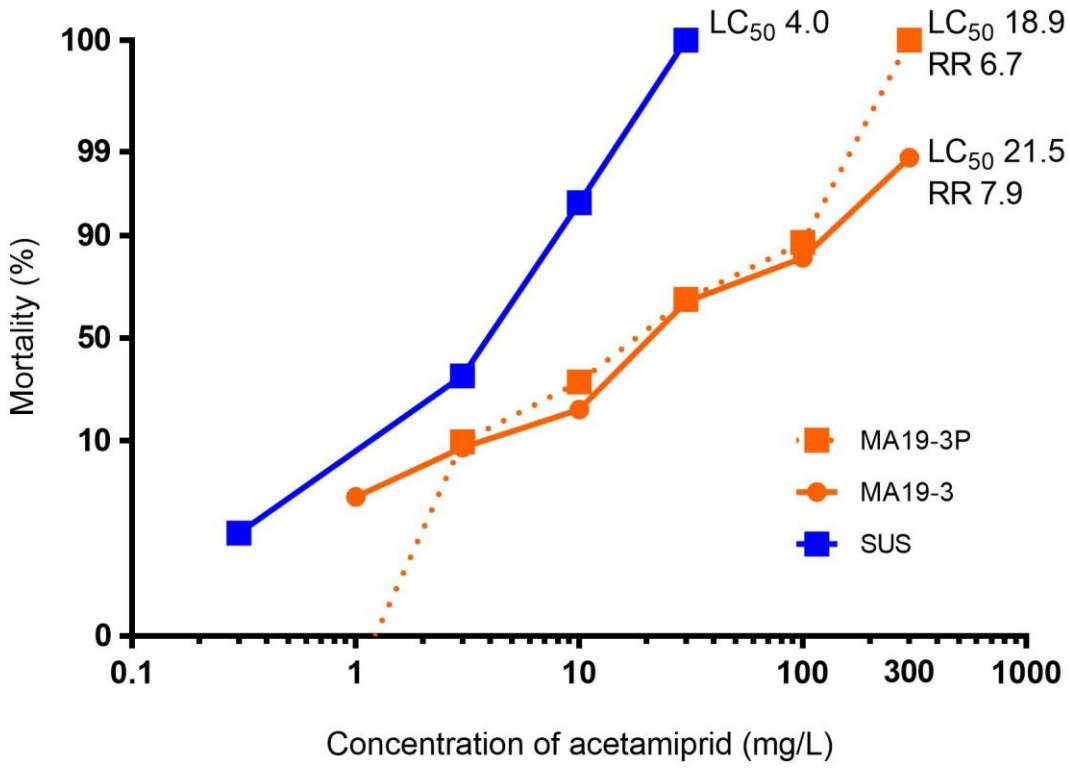

Figure 6. Dose response of MA19-3 to acetamiprid before and after exposure to 1000 mg/L 'pressuring' dose.

h) Emamectin benzoate

In total 50 populations were tested (Table 10). In the three years of testing. 100% mortality was recorded at doses of 30 mg/L or less (typically 10 mg/L) indicating populations are susceptible to emamectin benzoate.

Table 10. Bioassay summary data for emamectin benzoate 2016-17, 2017-18 and 2018-19

Season	Population	LC50 (mg/L a.i.)	Minimum Effective Conc. (mg/L a.i.)	Resistance Ratio (LC50)
2016-19	Susceptible (SU07-1)	0.9	30	-
2016-17	EM17-1	0.9	10	1.1
	TH17-1	1.1	10	1.2
	TH17-2	0.7	3	0.8
	DD17-1	0.8	0	1.0
	SG17-1	1.5	30	1.7
	SG17-2	0.6	3	0.7
	SG17-3	1.1	10	1.3
	MA17-1	0.6	3	0.7
	MA17-2	0.5	10	0.5
	MA17-3	0.7	10	0.8
	MO17-1	1.1	3	1.2
	MO17-2	1.0	3	1.1
	NM17-1	0.9	10	1.2
	NM17-2	0.6	10	0.7
	HI17-1	0.5	10	0.6
2017-18	EM18-1	1.6	10	1.8
	TH18-1	1.9	10	2.0
	SG18-1	1.4	10	1.5
	SG18-2	1.1	10	1.3
	SG18-3	0.8	10	0.9
	MA18-1	1.0	10	1.1
	MA18-2	0.8	10	0.8
	MA18-3	1.0	10	1.1
	MO18-1	1.7	10	1.8
	MO18-2	0.7	10	0.8
	MO18-3	0.8	10	0.9
	NM18-1	2.1	10	1.4
	NM18-2	1.3	10	2.3
	NM18-3	1.6	10	1.7
	WA18-1	0.6	10	0.6
	GR18-1	0.7	10	0.8
2018-19	EM19-1	0.8	3	0.9
	EM19-2	1.3	10	1.3
	TH19-1	0.8	10	0.9
	DD19-1	0.4	1	0.5
	SG19-1	1.1	10	1.3
	SG19-2	1.0	10	1.1
	SG19-3	1.1	10	1.2
	MU19-1	1.4	10	1.5
	MA19-1	1.2	10	1.4
	MA19-2	1.7	10	1.8
	MA19-3	0.7	10	0.7
	MO19-1	0.9	10	1.0
	MO19-2	0.7	3	0.7
	MO19-3	2.3	10	2.6
	NM19-1	1.1	10	1.3
	NM19-2	1.2	10	1.3
	NM19-3	1.2	10	1.3
	WA19-1	1.2	10	1.4
	GR19-1	0.7	10	0.8

i) Cyantraniliprole

Over the duration of the project, 33 populations were tested for resistance (Table 11). With the exception of SG19-1, all populations were effectively controlled at doses equal to or below the discriminating dose of 1 mg/L. In the case of SG19-1 a survivor was recorded at 1 mg/L, To test for resistance, SG19-1 was bioassayed again with a maximum dose of 3 mg/L, in this assay 100% mortality was achieved at 1 mg/L.

Table 11. Bioassay summary data for cyantraniliprole 2016-19.

Season	Population	LC50 (mg/L a.i.)	Minimum Effective Conc. (mg/L a.i.)	Resistance Ratio (LC50)
2016-19	Susceptible (SU07-1)	0.08	0.3	-
2016-17	EM17-1	0.02	0.3	0.3
	TH17-1	0.02	0.1	0.2
	TH17-2	0.02	0.1	0.3
	DD17-1	0.03	0.3	0.4
	SG17-1	0.04	0.3	0.6
	SG17-2	0.02	0.3	0.2
	SG17-3	0.06	1	0.7
	MA17-1	0.2	1	3.1
	MA17-2	0.02	0.3	0.3
	MA17-3	0.07	1	0.9
	MO17-1	0.04	0.1	0.5
	MO17-2	0.06	1	0.8
	NM17-1	0.02	0.3	0.3
	NM17-2	0.09	1	1.2
	HI17-1	0.03	0.3	0.3
2017-18	EM18-1	0.02	1	0.2
	TH18-1	0.04	1	0.5
	SG18-1	0.07	0.3	0.9
	MA18-3	0.04	1	0.5
	MO18-1	0.07	1	0.9
	NM18-3	0.05	1	0.6
	WA18-1	0.03	1	0.4
	GR18-1	0.05	1	0.6
2018-19	EM19-1	0.02	1	0.3
	TH19-1	0.3	1	0.4
	DD19-1	0.01	0.1	0.2
	SG19-1	0.03	-	0.4
	MU19-1	0.03	1	0.5
	MA19-2	0.03	1	0.4
	MO19-3	0.02	0.1	0.3
	NM19-3	0.02	0.1	0.2
	WA19-1	0.02	0.1	0.3
	GR19-1	0.04	1	0.5

j) Buprofezin

Over the course of the project baseline susceptibility data was gathered on 42 populations (Table 12). During 2016-17 and 2017-18, the highest minimum effective dose recorded was 100 mg/L. In 2018-19, populations from Emerald (EM19-1) and Goondiwindi (MA19-1, & 2) had survivors at 100 mg/L, when tested in follow up assays with doses between 10 and 300 mg/L all individuals were dead at 100 mg/L or lower. No cross-resistance to pyriproxyfen was found when the pyriproxyfen lab resistant strain (AY09-1R) was bioassayed with buprofezin (Figure 7).

Table 12. Bioassay summary data for buprofezin 2016-17, 2017-18 and 2018-19

Season	Population	LC50 (mg/L a.i.)	Minimum Effective Conc. (mg/L a.i.)	Resistance Ratio (LC50)
2016-19	Susceptible (SU07-1)	2.7	30	-
2016-17	EM17-1	2.2	100	1.7
	TH17-1	2.7	100	0.8
	DD17-1	1.6	100	1.7
	SG17-2	0.9	100	0.8
	MA17-1	1.5	100	0.2
	MO17-2	1.2	10	1.2
	NM17-1	1.8	100	1.2
	HI17-1	9.3	100	2.5
	Ay09-1R*	2.7	100	1.0
2017-18	EM18-1	1.3	100	0.5
	TH18-1	3.5	100	1.3
	SG18-1	1.5	100	0.6
	SG18-2	3.2	100	1.2
	SG18-3	1.8	100	0.7
	MA18-1	3.1	100	1.1
	MA18-2	2.0	100	0.7
	MA18-3	7.9	100	2.9
	MO18-1	5.3	100	6.4
	MO18-2	3.2	100	3.2
	MO18-3	4.0	100	5.5
	NM18-1	1.7	100	0.6
	NM18-2	1.7	100	0.6
	NM18-3	1.3	100	0.5
	WA18-1	4.1	100	1.5
	GR18-1	3.0	100	1.1
2018-19	EM19-1	5.6	-	2.0
	EM19-2	1.5	10	0.5
	TH19-1	3.1	100	1.3
	DD19-1	3.4	100	1.3
	SG19-1	1.9	100	0.7
	SG19-2	2.9	100	1.1
	SG19-3	3.5	100	1.3
	MU19-1	10.8	100	4.0
	MA19-1	11.6	-	4.2
	MA19-2	7.7	-	2.8
	MA19-3	4.4	100	1.6
	MO19-2	2.8	100	1.0
	MO19-3	1.6	100	0.6
	NM19-1	3.4	32	1.3
	NM19-2	5.5	100	2.0
	NM19-3	2.0	32	0.7
	WA19-1	6.2	100	2.3
	GR19-1	3.3	100	1.2

* pyriproxyfen resistant lab strain

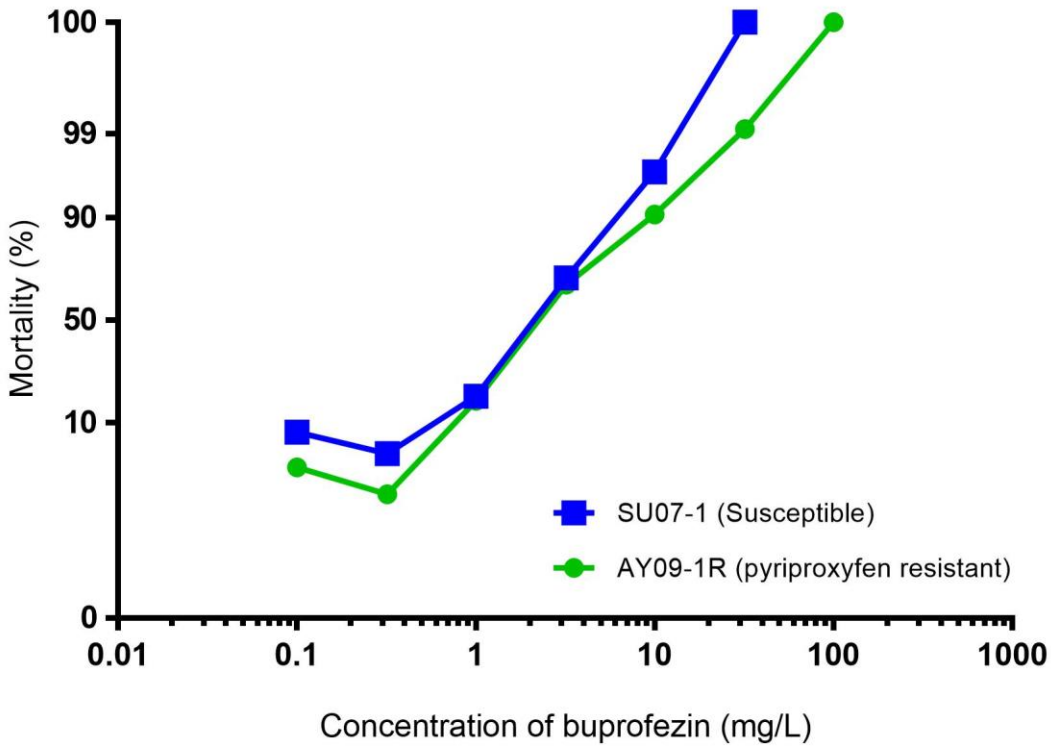

Figure 7. Dose response of a *Bemisia tabaci* pyriproxyfen resistant strain (AY09-1R) to buprofezin.

k) Afidopyropen

To determine the baseline level of susceptibility and screen for cross-resistance, bioassays were completed on 36 populations, including lab resistant populations (pyriproxyfen AY09-1R, spirotetramat AY16-1R and pyrethroid PB1510R) and a susceptible lab population SU07-1 (Table 13). Methodology was changed in 2018-19 to include the additive hasten, which increased the toxicity of afidopyropen. No evidence of cross-resistance between afidopyropen and pyriproxyyen, spirotetramat or pyrethroids was detected (Figure 8).

Table 13. Bioassay summary data for afidopyropen 2016-19.

Season	Population	LC50 (mg/L a.i.)	Minimum Effective Conc. (mg/L a.i.)	Resistance Ratio (LC50)
2017-18	Susceptible (SU07-1)	16.3	100	-
2018-19	Susceptible (SU07-1)	2.8	10	-
2017-18	TH18-1	18.7	300	1.1
	SG18-1	18.5	300	1.1
	SG18-2	18.1	300	1.1
	SG18-3	26.7	300	1.6
	MA18-1	16.6	100	1.0
	MA18-2	17.5	100	1.1
	MA18-3	21.6	300	1.3
	MO18-1	27.3	300	1.7
	MO18-2	20.8	300	1.3
	MO18-3	10.0	100	0.6
	NM18-1	22.2	300	1.4
	NM18-2	33.5	300	2.1
	NM18-3	14.9	100	0.9
	WA18-1	23.81	300	1.5
	GR18-1	17.1	300	1.1
	AY16-1R*	7.6	100	0.5
	AY09-1R#	8.0	300	0.5
	PB1510R^	12.8	300	0.8
2018-19	EM19-1	4.8	100	1.7
	EM19-2	3.9	100	1.4
	TH19-1	5.7	100	2.1
	DD19-1	5.2	100	1.9
	SG19-1	4.7	100	1.7
	SG19-2	3.6	100	1.3
	SG19-3	4.8	30	1.7
	MU19-1	4.0	100	1.5
	MA19-1	3.7	100	1.3
	MA19-2	4.9	100	1.8
	MO19-1	2.2	100	0.8
	MO19-2	2.1	100	0.8
	MO19-3	4.2	100	1.5
	NM19-1	10.2	100	3.7
	NM19-2	13.6	100	4.9
	NM19-3	18.0	100	6.5
	WA19-1	6.5	100	2.4
	GR19-1	3.8	100	1.4

* spirotetramat resistant lab strain
# pyriproxyfen resistant lab strain
^ pyrethroid resistant lab strain

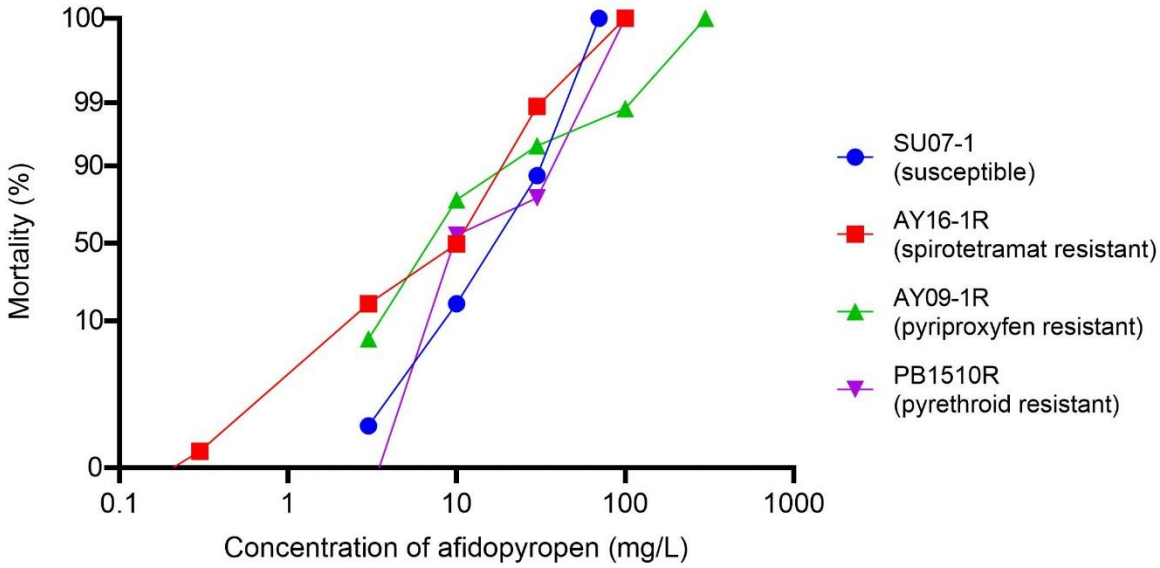

Figure 8. Dose response of *Bemisia tabaci* strains with resistance to spiroteramat, pyriproxyfen and pyrethroids to afidopyropen.

Impact of new insecticides on the whitefly parasitoid Eretmocerus hayati

Over the duration of the project two products were registered for silverleaf whitefly control. Skope® a co-formulation of acetamiprid (Group 4A) and emamectin benzoate (Group 6) was registered in 2017, while Versys® with the active afidopyropen (Group 9D) was registered in 2018.

In a laboratory experiment using a Potter tower (spray tower) we tested the toxicity of dried spray residue of six insecticides on *Eretmocerus hayati* (Figure 9.). Pyriproxyfen had the lowest toxicity throughout the experiment; while not significantly different from afidopyropen at 24 hours after treatment (HAT) it was different at 48 and 72 HAT (Table 14).

Afidopyropen was significantly less toxic than emamectin benzoate, acetamiprid and bifenthrin at each time after treatment. It was significantly less toxic than diafenthiuron at 48 and 72 HAT. Diafenthiuron was significantly less toxic than emamectin benzoate, acetamiprid and bifenthrin at 24 HAT, but at subsequent time periods there was no differences between these insecticides. At 24 HAT acetamiprid was significantly less toxic than bifenthrin, but not at 48 and 72 HAT.

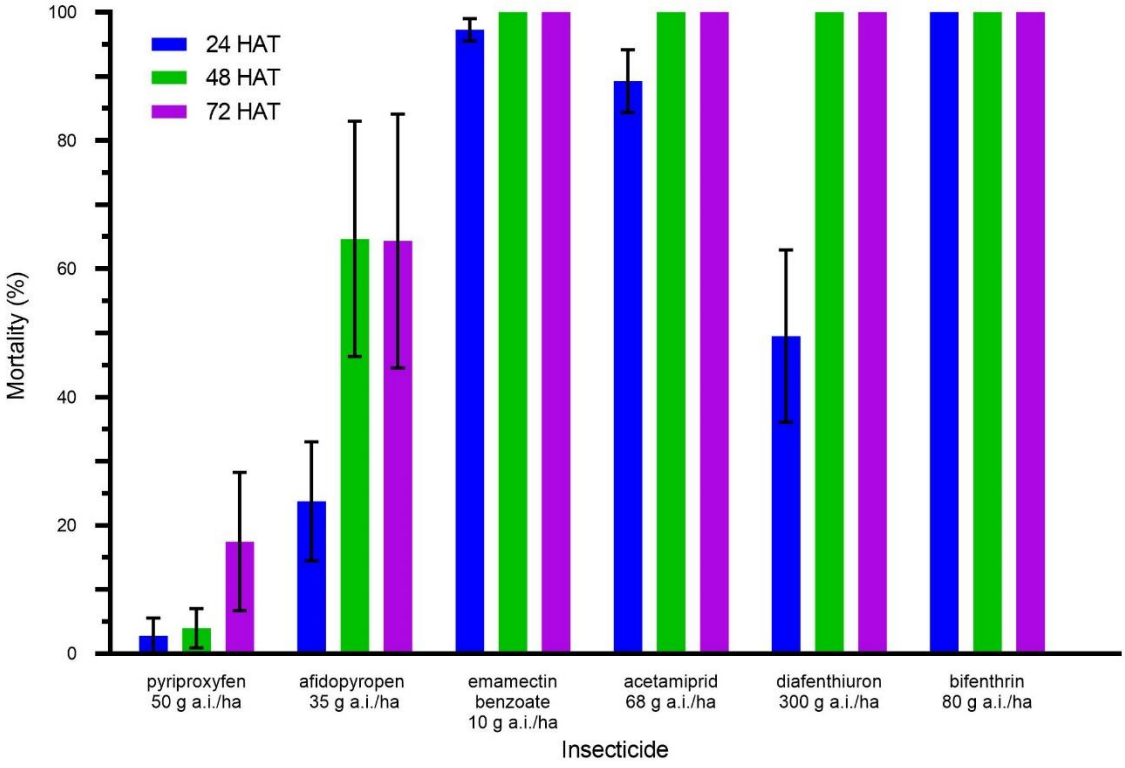

Figure 9. Mortality (± SEM) of *Eretmocerus hayati* at 24, 48 and 72 hours after exposure to dried residue of pyriproxyfen, emamectin benzoate, acetamiprid, afidopyropen, diafenthiuron and bifenthrin.

Table 14. Toxicity of 6 insecticides on *Eretmocerus hayati* at 24, 48 and 72 HAT.

Insecticide	Mortality (%)		
	24 HAT	48 HAT	72 HAT
pyriproxyfen	2.8 ± 2.8 d	4.0 ± 3.1 c	17.5 ± 17.5 c
afidopyropen	23.7 ± 9.3 cd	64.7 ± 18.4 b	64.3 ± 64.3 b
emamectin benzoate	97.3 ± 2.7 ab	100 ± 0 a	100 ± 0 a
acetamiprid	89.3 ± 4.9 b	100 ± 0 a	100 ± 0 a
diafenthiuron	49.5 ± 13.5 c	100 ± 0 a	100 ± 0 a
bifenthrin	100 ± 0 a	100 ± 0 a	100 ± 0 a

Means in the same column followed by the same letter are not significantly different in ANOVA, using LSD tests at P = 0.05.

The residual toxicity of afidopyropen, acetamiprid and emamectin benzoate on *E. hayati* was determined at 6 and 9 days after treatment. The experiment was originally designed to include 1, 2, 4, 8 and 12 days, but low emergence of *E. hayati* meant adjustments had to be made and only two time periods could be evaluated. Assessment were done at 24 and 48 h, but high control mortality at 48 h compromised that time period. Residual toxicity of the three insecticides was ≤20% at 6 days after application and ≤ 10% at 9 days, indicating the toxicity to *E. hayati* declines rapidly within a week of application (Figure 10). At both time periods there was no significant differences between the insecticides.

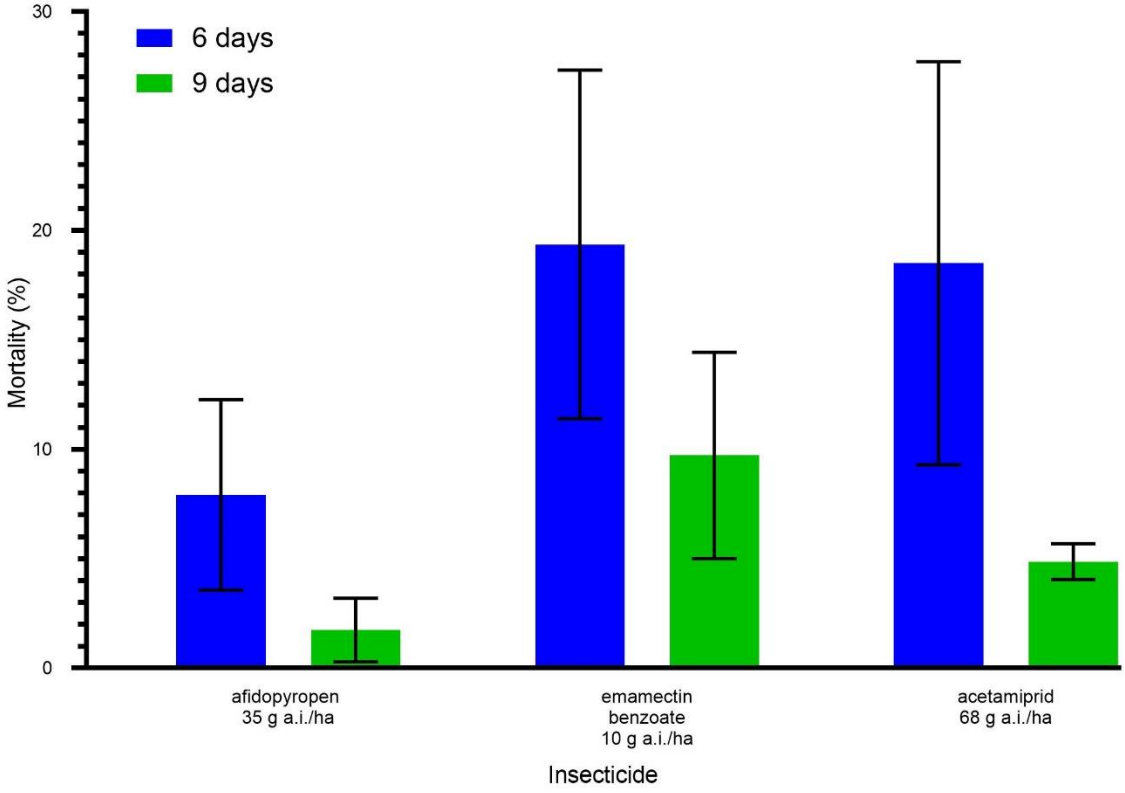

Figure 10. Mortality (± SEM) of *Eretmocerus hayati* at 24 h after exposure to 6 and 9 day old residue of afidopyropen, emamectin benzoate and acetamiprid.

Extension and reporting

Over the duration of this project research outputs were presented to industry at field days, seminars and in print media. Below is a list of extension activities.

Field days/workshops/seminars

- IPM workshops, Cecil Plains and Boggabilla, 30th November 2016
- CottonInfo Bug checking workshop, Boggabilla, 1st December 2016
- Silverleaf whitefly review meeting, Moree, 16th December 2016
- Insecticide resistance update, CCA seminar Dubbo, 19th May 2017
- Silverleaf whitefly insecticide resistance, CCA seminar Moree, 19th July 2017
- Area wide management meeting, Moree, 17th August 2017.
- CottonInfo IPM, pest and beneficial identification workshop, Boggabilla 29th November 2017
- Silverleaf whitefly (SLW) resistance status, Pakistan BCI tour, Toowoomba, April 2018
- A beneficial approach to whitefly, panel discussion, CCA seminar Narrabri, June 2018
- IPM specialist panel discussion and identification of whitefly parasitism, CCA workshops Moree, Dalby & Griffith, August – September 2018
- *Bemisia tabaci* insecticide resistance management in Australian cotton, 3rd international whitefly symposium, Fremantle 16-19th September 2018
- Area wide management meeting, Mungindi, 19th February 2019
- Area wide management meeting, Moree "Norwood" 14th March 2019
- Silverleaf whitefly and mealybug Cottoninfo meeting, Gunnedah and Narrabri 19th June 2019

Print media/web content

- Hopkinson J, Wilson L & Grundy P. (2017) Silverleaf whitefly: Embracing IPM to reduce resistance risk. Australian cottongrower
- Hopkinson J. (2017) Considerations when using pyriproxyfen on silverleaf whitefly. (http://thebeatsheet.com.au/pyriproxyfen-and-silverleaf-whitefly)
- Hopkinson J. (2017) Impact of insecticides on silverleaf whitefly parasitoid. (http://thebeatsheet.com.au/impact-of-insecticides-on-silverleaf-whitefly-parasitoid)
- Grundy P & Hopkinson J (2017) The challenges of cotton pest management during 2016/17 season. Cottoninfo webinar
- Hopkinson J. (2017) Monitoring tells resistance story. Spotlight
- Grundy P, Grundy T & Hopkinson J (2017) Silverleaf whitefly (SLW) in cotton: 5. How key pesticides work and minimising resistance (4:25) (https://youtu.be/UABalUkioYI)
- Hopkinson J, Grundy P & Grundy T (2017) Identifying parasitism (2:05) (https://youtu.be/SO0cedrGlQI)
- Hopkinson J (2017) Spirotetramat resistance update. Bayer webinar
- Hopkinson J & Ceeney S (2018) Silverleaf whitefly: insecticide resistance update. Australian cottongrower
- Hopkinson J (2018) Silverleaf whitefly: maintaining control. Spotlight

Journal publications

- Hopkinson, J. E., and Pumpa, S. M. (2019). Baseline susceptibility of *Bemisia tabaci* MEAM1 (Hemiptera: Aleyrodidae) in Australia to spirotetramat, cyantraniliprole and dinotefuran, with reference to pyriproxyfen cross-resistance. Austral Entomology (early access)

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.

1. By identifying which members of the *Bemisia tabaci* species complex were present in the populations collected in 2017, the project was able provide industry with confidence that the invasive *B. tabaci* MED is not present in Australian cotton.

2. Over the duration of the project, regular updates on whitefly resistance levels were provided to industry, including at annual TIMS insecticide technical panel meetings. Results and implications of resistance monitoring were presented widely within cotton industry at extension events.

3. Findings from the study of insecticide impacts on *Eretmocerus hayati* have been used to update the Cotton Pest Management Guide for 2019-20. Information from this study, earlier studies and complimentary international studies have been used to provide guidance to agronomists on IPM at extension events.

4. Results from this project, along with IPM messages have been delivered through a range of extension activities, for a full list see above (Results: Extension and reporting).

6. Please describe any:-

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

N/A

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

Project staff, developed methodology for single mated pair experiments, which could prove useful in future resistance or genetics studies.

c) required changes to the Intellectual Property register.

Established insect population (AY16-1R) resistant to spirotetramat

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?

This project has provided the necessary data and advice to industry via TIMS to make changes to the IRMS so resistance development to IPM-compatible insecticides is managed effectively.

While resistance to pyriproxyfen has become more widespread over the duration of this project, early intervention, with changes to the IRMS has reduced the severity of resistance, and based on data over three years, the instances and severity of resistance are in decline. Future monitoring will identify if this is a clear reversal or an aberration linked to seasonal variability in whitefly populations and product usage. This experience demonstrates the important service that yearly resistance monitoring delivers when coupled with an IRMS review process supported by industry.

The project team have supported Dow/Corteva in the registration of buprofezin by APVMA, providing baseline data on its toxicity against silverleaf whitefly. Pending approval, buprofezin should be available in the coming season. The discovery of spirotetramat resistance and subsequent international collaboration on the mechanism and mode of inheritance means the industry is well positioned and we have pre-emptively adjusted the IRMS to mitigate the risk posed. It will be important for the cotton industry to use spirotetramat in rotation with pyriproxyfen, buprofezin and other insecticides to minimise the development of resistance to spirotetramat.

During the project we evaluated the toxicity of acetamiprid, emamectin and afidopyropen on the whitefly parasitoid *E. hayati.* The results give an initial indication that afidopyropen may not be as selective as initially thought but more research needs to be undertaken in this area. Preliminary results have been included in the most recent Cotton Pest Management Guide. Methodology for handling and bioassaying *E. hayati* has been improved in this project.

An improved understanding of the distribution of silverleaf whitefly pyrethroid and organophosphate resistance in Australian cotton has been achieved by collaborating with the University of Canberra (Michael Frese, Grace Fang) and CSIRO (Tom Walsh, Wee Tek Tay). Grace completed an Honours study at the University of Canberra.

Whitefly are now a major pest in most growing regions, every year. Managing silverleaf whitefly to avoid sticky cotton is of great importance to the industry. The ongoing availability and effectiveness of pyriproxyfen, spirotetramat and buprofezin will provide cotton growers with IPM-compatible insecticides for silverleaf whitefly control.

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.

Ongoing research in this field has been funded, DAQ2001 – Sustainable SLW management through improved insect resistance monitoring. Methodology developed in DAQ1701 will be applied to DAQ2001.

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

Project outcomes, along with finding from ongoing research will contribute to the content of the cotton pest management guides, QDAF extension (Beatsheet,) CottonInfo and CRDC extension (Spotlight) and other industry extension (CCA, Australian cottongrower)

(c) for future research.

Future research was outlined in the project proposal for DAQ2001; resistance monitoring will continue to screen for resistant SLW in cotton. Baseline data gathered on acetamiprid, emamectin benzoate, buprofezin and afidopyropen will be reviewed and used to develop discriminating doses for DAQ2001. A review of available molecular screening techniques will be undertaken and extension activities focused on whitefly parasitism will be conducted with CottonInfo.

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)

1. Hopkinson, J. E., and Pumpa, S. M. (2019). Baseline susceptibility of Bemisia tabaci MEAM1 (Hemiptera: Aleyrodidae) in Australia to spirotetramat, cyantraniliprole and dinotefuran, with reference to pyriproxyfen cross-resistance. Austral Entomology (early access)
2. Hopkinson J, Pumpa S, van Brunschot S, Fang C, Frese M, Tek Tay W and Walsh T. Insecticide resistance status of Bemisia tabaci MEAM1 (Hemiptera: Aleyrodidae) in Australian cotton production valleys. Submitted to Austral entomology.

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

1. Two CottonInfo YouTube videos were made in relation to this project. Silverleaf whitefly (SLW) in cotton: 5. How key pesticides work and minimising resistance (4:25) (https://youtu.be/UABaIUkioYI) Identifying parasitism (2:05) (https://youtu.be/SO0cedrGlQI)
2. In collaboration with Garry McDonald and James Maino of the University of Melbourne a developmental model for *Bemisia tabaci* based on published literature has been added to Darabug https://jamesmaino.shinyapps.io/darabug2/.

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 internet. 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.

Over the three years of this project, data has been collected on the resistance status of silverleaf whitefly, *Bemisia tabaci* (MEAM1) to registered insecticides. In response to emerging resistance to the IGR pyriproxyfen, a recommendation to restrict usage of this IPM-compatible product to a 30 day window was adopted for the 2017/18 cotton season. This usage window has remained in place for subsequent seasons and testing indicates that resistance levels have stabilised. This research has been submitted for publication in Austral Entomology and is currently under review.

In 2016 resistance to spirotetramat was detected at two localities in North Queensland. Subsequent research has focused on understanding the underlying genetics of this resistance, and is in preparation for publication. In the most recent season (2018/19) resistance to spirotetramat was found in Emerald, which is the first record in a cotton production region. In response, the IRMS has been changed to restrict the usage of this insecticide to a single use per field (except for fields treated for mealybug which require a double application as per label direction to be effective).

Resistance to acetamiprid was suspected in the Macintyre region near Goondiwindi after the first round of bioassay testing. Further testing couldn't confirm resistance, suggesting an initial false positive result.

Over the duration of the project, baseline susceptibility testing of products entering the cotton marketplace for control of silverleaf whitefly has been completed. This includes buprofezin, acetamiprid, emamectin benzoate and afidopyropen. A manuscript documenting earlier testing of products including spirotetramat, cyantraniliprole and dinotefuran was published in Austral Entomology.

In response to the widespread outbreaks of silverleaf whitefly, particularly in the 2016/17 season, the project team has actively engaged in extension events facilitated by the CCA and CottonInfo. This has included the production of videos on both resistance monitoring and whitefly parasitism.

Interest in assessing whitefly parasitism has grown steadily over the course of the project and knowledge on how to assess parasitism was presented at workshops run by the CCA at Moree, Dalby and Griffith. Evaluation of the toxicity of newer products against *Eretmocerus* was undertaken, but further experiments are needed to confirm results before this research can be confidently extended to industry.

The widespread extension of whitefly management issues, including stickiness, resistance and parasitism means cotton agronomists are better informed on the threat whitefly poses and the critical role IPM will play going forward.

Jamie Hopkinson
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Appendix 1. Collection details for whitefly samples (2016–2019)

	Site code	Host plant	Locality	Collection date
2016-17	AY16-1	rock melon	Ayr	28/07/16
	BO16-1	tomato	Bowen	28/07/16
	EM17-1	cotton	Emerald	1/02/17
	TH17-1	cotton	Theodore	2/02/17
	TH17-2	cotton	Theodore	2/02/17
	DD17-1	cotton	Darling Downs	9/03/17
	SG17-1	cotton	St George	16/02/17
	SG17-2	cotton	St George	16/02/17
	SG17-3	cotton	St George	16/02/17
	MA17-1	cotton	Goondiwindi	3/03/17
	MA17-2	cotton	Goondiwindi	3/03/17
	MA17-3	cotton	Goondiwindi	3/03/17
	MO17-1	cotton	Moree	29/03/2017
	MO17-2	cotton	Moree	29/03/2017
	MO17-3	cotton	Moree	16/05/2017
	MO17-4	cotton	Moree	9/06/2017
	MO17-5	sowthistle	Moree	4/08/2017
	MO17-6	sowthistle	Garah	7/08/2017
	NM17-1	cotton	Narrabri	29/03/17
	NM17-2	cotton	Narrabri	29/03/17
	HI17-1	cotton	Hillston	17/03/17
2017-18	EM18-1	cotton	Emerald	30/01/2018
	TH18-1	cotton	Theodore	31/01/2018
	SG18-1	cotton	St George	1/03/2018
	SG18-2	cotton	St George	1/03/2018
	SG18-3	cotton	St George	1/03/2018
	MA18-1	cotton	Goondiwindi	15/03/2018
	MA18-2	cotton	Goondiwindi	15/03/2018
	MA18-3	cotton	Goondiwindi	15/03/2018
	MO18-1	cotton	Moree	14/03/2018
	MO18-2	cotton	Moree	14/03/2018
	MO18-3	cotton	Moree	14/03/2018
	NM18-1	cotton	Narrabri	12/03/2018
	NM18-2	cotton	Narrabri	12/03/2018
	NM18-3	cotton	Narrabri	12/03/2018
	WA18-1	cotton	Warren	6/02/2018
	GR18-1	cotton	Griffith	10/05/2018
2018-19	EM19-1	cotton	Emerald	22/01/19
	EM19-2	cotton	Emerald	30/01/19
	TH19-1	cotton	Theodore	21/01/19
	DD19-1	cotton	Darling Downs	1/03/19
	SG19-1	cotton	St George	6/03/19
	SG19-2	cotton	St George	6/03/19
	SG19-3	cotton	St George	6/03/19
	MU19-1	cotton	Mungindi	1/03/19
	MA19-1	cotton	Goondiwindi	11/03/19
	MA19-2	cotton	Goondiwindi	11/03/19
	MA19-3	cotton	Goondiwindi	11/03/19
	MO19-1	cotton	Moree	15/03/19
	MO19-2	cotton	Moree	15/03/19
	MO19-3	cotton	Moree	15/03/19
	NM19-1	cotton	Narrabri	21/03/19
	NM19-2	cotton	Narrabri	21/03/19
	NM19-3	cotton	Narrabri	21/03/19
	WA19-1	cotton	Warren	15/04/19
	GR19-1	cotton	Griffith	1/04/19