FINAL REPORT

CRDC ID: DAQ2001

Project Title: Sustainable SLW management through improved insect resistance monitoring

Confidential or for public release? For Public Release

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Part 1 – Contact Details & Submission Checklist

Principal Researcher: **Dr Jamie Hopkinson, Senior research scientist**
Organisation: Department of Agriculture and Fisheries, Queensland Government
Ph: 0475 825 340
E-mail: jamie.hopkinson@daf.qld.gov.au

Supervisor: **Dr Paul Grundy, Principal research scientist**
Organisation: Department of Agriculture and Fisheries, Queensland Government
Ph: 0427 929 172
E-mail: paul.grundy@daf.qld.gov.au

Researcher 2:
Organisation:
Ph:
E-mail:

Submission checklist.

Please ensure all documentation has been completed and included with this final report:

☐ Final report template (this document)
☐ Final Technical Report
☐ Final Schedule 2: IP register
☐ Final Schedule 3: Acknowledgment
☐ Final financial report
☐ PDF of all journal articles (for CRDC's records)

Signature of Research Provider Representative: _____

Date submitted: _____

Part 2 - Monitoring & Evaluation

Achievement against milestones in the Full Research Proposal

Milestone	Progress	Explanation
1. Biosecurity surveillance	Achieved	Whitefly species identification completed on samples from 2015-2021 Whitefly samples from 2022 in ethanol awaiting DNA extraction – results will be reported during DAQ2301
2. Resistance monitoring of silverleaf whitefly	Achieved	Insecticide bioassays testing resistance levels of whitefly completed
3. Parasitism assessment	Achieved	CottonInfo Regional Extension Officers in three regions skilled in whitefly parasitism assessment
4. Review of molecular-based detection of resistance in silverleaf whitefly	Achieved	Review completed and submitted to CRDC with final report

Outputs produced.

Output	Description
Reports	1. Final report, *Sustainable SLW management through improved insect resistance monitoring,* September 2022. ○ Literature review, *Molecular-based detection of resistance in Bemisia tabaci.*
Publications	1. Fang, C., Hopkinson, J. E., Balzer, J., Frese, M., Tay, W. T., and Walsh, T. (2022). Screening for insecticide resistance in Australian field populations of Bemisia tabaci (Hemiptera: Aleyrodidae) using bioassays and DNA sequencing. Pest Management Science 78, 3248-3259. 2. Hopkinson, J., Pumpa, S., van Brunschot, S., Fang, C., Frese, M., Tay, W. T., and Walsh, T. (2020). *Insecticide resistance status of Bemisia tabaci MEAM1 (Hemiptera: Aleyrodidae) in Australian cotton production valleys.* Austral Entomology 59, 202-214. 3. Lueke, B., Douris, V., Hopkinson, J. E., Maiwald, F., Hertlein, G., Papapostolou, K. M., Bielza, P., Tsagkarakou, A., Van Leeuwen, T., Bass, C., Vontas, J., and Nauen, R. (2020). *Identification and functional characterization of a novel acetyl-CoA carboxylase mutation associated with ketoenol resistance in Bemisia tabaci.* Pesticide Biochemistry and Physiology 166.
Presentations	1. *Insecticide resistance status of Bemisia tabaci MEAM1,* Australian cotton research conference, Armidale. October 2019. 2. *Lessons from consumption studies with predators,* Natural enemy workshop, Virtual Workshop. May 2020. 3. *Update on SLW insecticide resistance,* 2020 CSD cotton management tour. Virtual forum. July 2020. 4. *Update on SLW insecticide resistance,* The Australian Cottongrower virtual forum. August 2020.

Extension articles	1. Hopkinson, J., Herron, G., and Grundy, P. (2019) *Resistance update – mites, aphids, thrips, mirids and SLW,* Australian Cottongrower 40, October-November p40-41. 2. *SLW resistance remains,* Spotlight on Cotton R&D, Winter 2022 p27-28. 3. Bird, L., Hopkinson, J., and Grundy, P. (2020) *Resistance update – mites, aphids, helicoverpa, mirids and SLW,* Australian Cottongrower 41, October-November p14-16. 4. Bird, L., Hopkinson, J., and Grundy, P. (2021) *Resistance update – mites, aphids, helicoverpa, mirids and SLW,* Australian Cottongrower 42, October-November p14-16. 5. *Insects: Managing Silverleaf Whitefly in Australian cotton,* CottonInfo Factsheet. December 2020. 6. *Resistance testing 2020-21 Season,* CottonInfo factsheet. November 2021.
Extension events	1. *Cotton Catchup.* CottonInfo, Dalby, November 2019. 2. *Cotton IPM and Bugchecking.* Dalby & Goondiwindi, December 2021.

Outcomes from project outputs.

Outcome	Description
Adoption of research findings	1. Changes made to cotton IRMS, annually restricting spirotetramat and buprofezin to single applications per field for silverleaf whitefly control. 2. Ongoing use of 30-day spray window for pyriproxyfen by cotton industry. 3. Hopkinson et. al. (2020) has been cited 9 times, Lueke et. al. (2020) has been cited 14 times.
Collaboration	1. Collaboration between DAF, CSIRO, and University of Canberra has resulted in publication of 2 journal articles (see above) and other research that is in preparation for publication. 2. Collaboration with Bayer AG, IMBB/FORTH, University of Crete, Cartagena Polytechnical University, Hellenic Agricultural Organisation, Ghent University, University of Exeter, and University of Athens, resulted in publication of 1 journal article. 3. CottonInfo, whitefly parasitism extension to industry, Border rivers, St George & Dirranbandi, Gywdir & Mungindi, and Macquarie & Bourke.

Part 3 – Technical Report

Contents

Executive summary

Although it has been several years since a major outbreak of silverleaf whitefly (SLW), the ongoing risk of honeydew contamination means they remain a pest of concern for growers in almost all cotton production valleys. Control of SLW still relies heavily on the use of insecticides, although biological control, including conserving natural populations and augmentation via parasitoid wasp releases, has been adopted by some growers.

Silverleaf whitefly resistance to pyriproxyfen emerged as a significant issue during the 2016/17 season. The industry responded by introducing a 30-day application 'spray' window to restrict pyriproxyfen use, aiming to reduce resistance selection pressure. From 2018 to 2020 this approach showed promise with a steady decline in the number of populations carrying resistance. However, in 2021 and 2022 close to half of the tested populations contained some resistant individuals. While the severity of resistance is lower than that observed in 2016/17, it is still a concerning reversal of the earlier trajectory.

Within the cotton industry, detection of SLW resistance to spirotetramat was first documented in Emerald in 2019. During the last three seasons, spirotetramat resistance was detected in populations collected from cotton in Emerald, Theodore, Darling Downs, Mungindi, and Macintyre and Namoi valleys. DNA sequencing of populations collected between 2019 and 2021 found the frequency of the mutation in resistant populations was low (1.2-4.1%). While current resistance levels are unlikely to cause management issues, SLW resistance to spirotetramat has the potential to increase (as already observed in other industries). A proactive change to the Insecticide resistance management strategy (IRMS) was made in 2019, reducing spirotetramat use to a single spray per field for SLW control.

A change in registration that occurred in 2020 now means buprofezin can be used in cotton for SLW control. Testing by bioassay has found no evidence of SLW resistance to buprofezin. Testing of laboratory strains with known resistance to pyriproxyfen and spirotetramat showed no signs of cross resistance to buprofezin. At the time of its inclusion in the IRMS, buprofezin was restricted to a single application when targeting SLW.

Widespread SLW resistance to imidacloprid and pymetrozine was found during testing in 2020. These results from 2020 and testing of laboratory neonicotinoid-resistant strain against a range of neonicotinoids show there is low-level cross resistance between imidacloprid and acetamiprid but no cross resistance to dinotefuran. Testing from 2020 to 2022 showed no definitive evidence of resistance to either acetamiprid or dinotefuran.

Resistance to the pyrethroid bifenthrin has been detected in many of the tested SLW populations over the past three years. This data, along with DNA sequencing data was recently published (Pest management Science, 78 – issue 8, August 2022,). Resistance to bifenthrin is widespread in cotton production regions, but within a population the mutation's frequency is generally low (1-7%). In the same study, we documented that *Bemisia tabaci* Middle East-Asia Minor 1 (formerly B-biotype) is the dominant species found in cotton grown in NSW and QLD. The potentially invasive *B. tabaci* Mediterranean (formerly Q-biotype) was not detected in any of the samples.

Extension of project findings was circulated primarily via annual updates in the *Australian cottongrower*, via CottonInfo factsheets and by CottonInfo's regional extension officers (REOs). Extension of SLW parasitism was achieved by engaging REOs in an in-field assessment of parasitism levels. The REOs gained firsthand experience and increased recognition of the value of biological control from parasitism during the exercise, which has been shared at a local level with agronomists.

Introduction

The cotton insect pest *Bemisia tabaci* Middle East-Asia Minor 1 (MEAM1), commonly known as silverleaf whitefly (SLW) is widely distributed in Australia. *B. tabaci* MEAM1 is an invasive pest that was first detected in Australia in 1994. It is part of a species complex (morphologically indistinguishable species), that could make the arrival of other invasive species like *B. tabaci* Mediterranean (MED) difficult to detect. Globally, *B. tabaci* MEAM1 is a vector of several important viruses of economic plants including cotton leaf curl disease.

In Australian cotton, SLW are primarily a pest due to their potential to contaminate cotton lint with honeydew, leading to reduced quality. High levels of stickiness incur significant price discounts and can lead to rejection by the buyer. Honeydew present on cotton lint also attracts the growth of sooty mould, which discolours lint. Cotton is graded on colour, with small premiums paid for good grades, but there can be significant discounts for poor grades.

Insecticide resistance in SLW is well documented. It makes management more difficult and has the potential to increase the cost of control. While several insecticides are registered for SLW control, in reality only a select few are widely used due to concerns regarding efficacy, detrimental impact on natural enemies, or potential of field failure due to resistance.

To manage the risk of resistance, SLW are included in the industry's insecticide resistance management strategy (IRMS). The strategy uses a combination of product windows and use restrictions to minimise the selection of resistance in pest populations. As insect populations can rapidly evolve resistance, this project collected annual data on the resistance status of SLW

obtained from several major cotton production regions. Based on observed changes in resistance levels, the data is used to update the IRMS and maintain its effectiveness.

The project also conducted biosecurity surveillance of the *B. tabaci* species complex present in cotton. Invasive whitefly, particularly, *B. tabaci* MED pose a significant biosecurity threat to Australia's cotton industry. Further incursions of *B. tabaci* have the potential to introduce new forms of resistance or diseases, like cotton leaf curl.

Biological control of silverleaf whitefly due to predation and parasitism is an important component of population suppression. This includes the activity of the parasitoid *Eretmocerus hayati*, which often goes unnoticed due to their minute size. To extend the skill of identifying parasitised whitefly nymphs this project collaborated with CottonInfo regional extension officers to share knowledge that could be disseminated to growers and agronomists in their respective regions.

Materials and methods

Biosecurity surveillance

To determine the species composition of whitefly present in Australian cotton, annual surveillance was completed across a number of cotton production regions. Surveyed regions included Emerald, Theodore, Darling Downs, St George, Mungindi, Goondiwindi, Moree, Narrabri, Warren, Hillston, and Griffith. From these collections, adult whiteflies were preserved in 90% ethanol and stored at -20°C. Preserved insect samples were sent to CSIRO for DNA extraction and sequencing.

DNA was extracted from the whitefly using the QIAGEN DNeasy Blood and Tissue kit following the manufacturer's instructions. Amplicons were generated using modified gene-specific primers, attached to Illumina linker sequences (Fang et al., 2022). The mtCOI barcoding region was amplified using Wfly-PCR-F1/RI and Wfly-PCR-F2/R2 primers (Table 1). All PCRs were performed using Platinum Taq and mtCOI gene sequences were amplified as described in Tay et al. (2022). Sanger sequencing was used for smaller sample sizes (n<30), while for larger populations (n>30) metabarcoding and high throughput sequencing was used for pooled whitefly samples.

All amplicon sequencing analysis was completed using CLC Genomics Workbench V21.0. Fastq files were imported as joint-paired-end reads and quality trimmed with 0.05 quality scores. Trimmed mtCOI reads were mapped to the updated *B. tabaci* mtCOI database (Kunz et al., 2019).

Table 1. Primer sequences used for *Bemisia* cryptic species identification

Primer name	Primer sequence	Amplicon size
Wfly-PCR-F1	TGGTTYTTTGGTCATCCRGAAG	645 bp
Wfly-PCR-R1	GGAAARAAWGTTAARTTWACTCC	
Wfly-PCR-F2	CGRGCTTAYTTYACTTCAGCYAC	663 bp
Wfly-PCR-R2	GGYTTATTRATTTTYCAYTCTA	

Resistance monitoring for silverleaf whitefly

Insects and plants

Presence of insecticide resistance in populations of silverleaf whitefly collected from cotton production regions of NSW and QLD was determined annually. Adult whiteflies were collected from commercial fields using a handheld vacuum (Stihl BG75) and from these collections, discrete populations were established and maintained under glasshouse conditions. Additionally, a small

number of populations were established from leaf collections sent to laboratory by agronomists and cottoninfo regional extension officers.

Cotton variety, Sicot 714B3RF (without insecticide seed treatment) was used for all insect rearing and insecticide bioassays. Plants were grown in small pots with potting mix. For the initial 3-4 weeks of growth, plants were reared under artificial light in a controlled environment (CE) room (29°C; 70% RH; 16L:8D). Once plants had reached between 4 and 6 true leaves, they were transferred from the CE room to a glasshouse where they were kept in large insect-proof cages for a further 3-4 weeks of growth. To minimise pest mite, aphid and thrips populations in the glasshouse, plants were kept for no longer than 8 weeks.

Bioassays

Silverleaf whitefly populations were tested for the presence of resistance to insecticides currently registered for their control. The life stage of whitefly tested in the assay was determined by the mode of action of the insecticide tested and assays were either systemic uptake or foliar leaf dip (Hopkinson et al., 2020; Hopkinson and Pumpa, 2019).

Egg assay – pyriproxyfen

The bioassay for the presence of pyriproxyfen resistance tested egg mortality and used a foliar leaf dip. A dosage range of 0.001 to 10 mg/L was used with the discriminating dose set at 10 mg/L, to distinguish between susceptible and resistant populations. A typical assay had 6 treatments, a control of diluent only (100 mg/L Agral), with 30–50 individuals per experimental unit and was replicated 5 times. An assessment of egg mortality was made at 10 days after treatment by counting both eggs that failed to hatch and nymphs that died during emergence.

Egg/nymph assay – cyantraniliprole

A systemic uptake assay was used to test the susceptibility of whitefly to cyantraniliprole. A dosage range of 0.01 to 1 mg/L was used with 5 treatment doses and a control of diluent only (distilled water). Assays were replicated 5 times with 30 insects per experimental unit. Uptake of cyantraniliprole was via the leaf petiole and treatment commenced 1 day after egg lay, with an assessment of mortality made on early instar nymphs at 14 days after treatment. A discriminating dose for cyantraniliprole was set at 1 mg/L.

Nymph assay – spirotetramat and buprofezin

A foliar leaf dip assay targeting 1st and 2nd instar nymphs was used to test the susceptibility of whitefly populations to spirotetramat and buprofezin. For spirotetramat the dosage range was 1 to 100 mg/L with 5 treatment doses, while for buprofezin a dosage range of 0.1 to 32 mg/L was used with 6 treatment doses. Both assays included a control of diluent only (100 mg/L Agral) and were replicated 5 times with 25-30 insects per experimental unit. For spirotetramat, the discriminating dose was set at 100 mg/L, while for buprofezin the discriminating dose is still to be determined, most likely it will be 200 mg/L.

Adult assay

For the remaining insecticides an adult assay was used to test the susceptibility of whitefly (Table 2). Each assay had 5 replicates and depending on insecticide, 5 to 7 treatment doses were tested and a control of diluent only was included. For adult assays, 20 to 25 insects are tested per experimental unit. In most cases these assays were foliar (leaf dip assays). Imidacloprid was tested using a systemic uptake, while flupyradifurone was tested using both foliar and systemic uptake.

Data analysis

Non-pooled bioassay data were analysed by probit (Genstat 19th edition). From this analysis lethal concentration estimates (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 (MEC) was recorded. For each population, a resistance ratio (RR) was derived by dividing the calculated LC_{50} of the field collected populations by the value of a reference susceptible population (SU07-1).

Table 2. Summary of bioassay methodology used for tested insecticides

Insecticide	Trade name	MoA	Dose range (mg/L a.i.)	Bioassay type	Development stage targeted	Duration (days)
Bifenthrin	Talstar®	3A	1 - 1000	foliar	adults	2
Acetamiprid	Intruder®	4A	1 - 300	foliar	adults	3
Dinotefuran	Starkle®	4A	1 - 320	foliar	adults	3
Imidacloprid	Confidor®	4A	0.1 - 100	systemic	adults	3
Flupyradifurone	Sivanto®	4D	0.01 - 1000	foliar & systemic	adults	4
Emamectin benzoate	Affirm®	6	0.1 - 10	foliar	adults	3
Pyriproxyfen	Admiral®	7C	0.001 - 10	foliar	eggs	10
Pymetrozine	Chess®	9B	3 - 1000	foliar	adults	4
Afidopyropen	Versys®	9D	1 - 100	foliar	adults	4
Diafenthiuron	Pegasus®	12A	0.3 - 100	foliar	adults	3
Buprofezin	Applaud®	16	0.1 - 32	foliar	2nd instar nymphs	11
Spirotetramat	Movento®	23	1 - 100	foliar	2nd instar nymphs	11
Cyantraniliprole	Exirel®	28	0.01 - 1	systemic	1st instar nymphs	13

Parasitism assessment

The ability to categorise whitefly nymphs as healthy or parasitised is used to determine field parasitism rates and is considered a valuable skill by many agronomists, so hands-on demonstrations were provided at a series of Crop Consultants Australia (CCA) workshops in 2018. Interest in whitefly parasitism resulted in the development of an extension exercise to build capacity amongst CottonInfo Regional Extension Officers (REOs), with the aim to provide agronomists in most regions with local support to foster the development of their own skills.

In 2019, CottonInfo regional agronomy staff purchased stereo microscopes with digital cameras so they could aid local agronomists and growers with crop protection related enquires.

To gain skill in parasitism identification, REOs collected 100 mid canopy leaves weekly for 3-5 weeks from a field in their region and recorded the number of healthy and parasitised whitefly on each leaf. If leaves were heavily infested, a representative section of leaf was used. Data was entered into Excel and the percentage of parasitised nymphs was calculated. Cloud-based storage was used to store images within the group, enabling shared learning. Features in the nymph photos could be highlighted to help learn the major diagnostic features used to identify whitefly parasitism.

Review of molecular-based detection of insecticide resistance in silverleaf whitefly

Literature review

Academic literature covering molecular-based studies of insecticide resistance relevant to *B. tabaci* and insecticides currently registered for its management were reviewed to gain a better understanding of how genotyping could be incorporated into current and future projects. Literature searches were conducted from 2019 to 2022 using Scopus, ScienceDirect, Web of Science and Google Scholar.

DNA sequencing

Populations of *B. tabaci* collected between 2013 and 2021 were sub-sampled into ethanol. DNA was extracted and sequenced for the presence of known resistance mutations for organophospates (F331W), pyrethroids (L925I). For the mutations A2083V and A2151V linked to spirotetramat, the same process was repeated but restricted to samples collected between 2017

and 2021. Sequencing data for pyrethroid and spirotetramat were compared to phenotypic data from bioassays. Full methodology of the organophosphate and pyrethroid study is published in (Fang et al., 2022), while the spirotetramat study is currently in preparation for publication, but Leuke et al. (2020) contains an earlier description of the methodology.

Results

Biosecurity surveillance

Whitefly collected between 2013 and 2021 primarily from 11 cotton production regions were identified to species level. From 144 field populations only two had a mixed species complex of AUS I and MEAM1; the remaining 142 populations consisted of MEAM1 only. The potentially invasive *B. tabaci* MED (formerly Q – biotype) was not detected, nor was ASIA II that has recently been reported as present in Australia (Wongnikong et al., 2021). The two populations with mixed AUS1 and MEAM1 were collected from Goondiwindi in 2016 (19.6% AUS I) and 2017 (7.9% AUS I). Over the past three years some populations were started from only a small field population (Narromine 2020), or failed to establish:

- Macintyre (1) 2021 failed to establish after collection
- Macintyre (4) 2021 slow to establish, only tested with pyriproxyfen
- Moree (1) 2022 failed to establish, very slow development time
- Moree (2) 2022 failed to establish, very slow development time

Resistance monitoring for silverleaf whitefly

Bioassays, that test the phenotypic response of field collected populations were completed for each of the insecticides registered for whitefly management. For some registered insecticides, testing was reduced, reflecting their limited use in cotton. For insecticides where discriminating doses have been developed, they were used to identify resistant populations. In the absence of a defined discriminating dose, resistance ratios were used to infer resistance. Resistance ratios represent the difference between a field population and a lab susceptible strain (LC_{50} of field population/LC_{50} of susceptible strain). The level of resistance is classified as; susceptibility (RR=1), tolerance to low resistance (RR = 2–10), moderate resistance (RR = 11–30), high resistance (RR = 31–100), very high resistance (RR > 100). In the absence of supporting evidence, low level resistance is difficult to infer from resistance ratios.

3A – Pyrethroids

Bifenthrin

Based on survival at a discriminating dose of 320 mg/L, resistance to bifenthrin was frequently detected in many of the populations tested (Table 3). While resistance is widespread, the frequency of the *vgsc* mutation *L925I* within the resistant populations from cotton production is low (1.1-4.7%). The resistance ratios calculated from the bioassay data are low, which is consistent with the molecular findings and suggests the target site mutation is the primary source of resistance in the tested populations.

Table 3. Summary of bifenthrin bioassay results

Population	Year	LC_{50} (mg/L)	(95% FL)	Slope (SE)	Mortality (%) at discriminating dose (320 mg/L)	Resistance Ratio*
Susceptible	-	2.99	(2.41-3.65)	2.53 (0.26)	100	-
Emerald	2020	9.29	(3.92-18.18)	0.93 (0.13)	93.7	3.1
Theodore	2020	9.90	(6.35-14.93)	1.30 (0.13)	97.6	3.3
Darling Downs	2020	4.75	(2.30-8.42)	0.99 (0.12)	95.0	1.6
St George (3)	2020	5.28	(2.90-8.82)	1.21 (0.16)	98.1	1.8
North Star	2020	5.11	(2.43-9.26)	1.13 (0.16)	93	1.7
Moree (3)	2020	3.76	(1.87-6.48)	0.96 (0.11)	98.1	1.2
Namoi valley (2)	2020	9.90	(6.53-14.31)	0.99 (0.08)	94.2	3.3

Population	Year	LC_{50} (mg/L)	(95% FL)	Slope (SE)	Mortality (%) at discriminating dose (320 mg/L)	Resistance Ratio*
Narromine	2020	5.26	(3.18-8.23)	1.46 (0.18)	98.5	1.76
Hillston	2020	7.94	(3.86-14.90)	1.34 (0.21)	96.4	2.66
Emerald	2021	5.24	(2.06-10.61)	0.76 (0.10)	88.5	1.8
Theodore	2021	3.77	(2.93-4.80)	2.07 (0.21	100	1.3
St George (2)	2021	8.84	(5.14-14.11)	1.02 (0.10)	89.7	3.0
Macintyre (2)	2021	10.69	(4.47-21.12)	0.85 (0.12)	86.2	3.6
Moree (3)	2021	3.81	(1.52-7.56)	1.10 (0.18)	97.5	1.3
Namoi valley (3)	2021	5.23	(2.60-9.21)	0.96 (0.12)	96.2	1.8
Narromine	2021	2.67	(1.22-4.82)	1.12 (0.17)	100	0.9
Hillston	2021	6.64	(2.84-12.91)	1.06 (0.16)	96.6	2.2
Griffith	2021	6.75	(3.31-12.05)	1.00 (0.13)	94.4	2.3
Theodore	2022	6.54	(3.66-10.80)	1.10 (0.12)	98.7	2.2
St George (2)	2022	10.84	(6.07-17.9)	1.04 (0.11)	89.9	3.6
Mungindi (1)	2022	3.57	(2.84-4.44)	3.21 (0.33)	100	1.2
Macintyre (1)	2022	1.85	(0.61-3.90)	0.77 (0.10)	98.8	0.6
Moree	2022	6.69	(3.34-12.28)	1.33 (0.20)	94.6	2.2
Namoi valley (3)	2022	13.88	(10.76-17.75)	1.61 (0.12)	100	4.6

***** Resistance ratio is calculated as (LC_{50} of field population/LC_{50} of lab susceptible population)

4A – Neonicotinoids & 9B Pymetrozine

Over the duration of the project, silverleaf whitefly were tested for resistance to several neonicotinoids including imidacloprid, acetamiprid and dinotefuran. Cross-resistance within the neonicotinoid group and between neonicotinoids and pymetrozine is known to occur (Elbert and Nauen, 2000; Nauen et al., 2013) and for this reason field-collected populations in 2020 were tested to determine levels of resistance to imidacloprid and pymetrozine. To further investigate cross-resistance, the response of lab-resistant strain (GR15-1R) to several neonicotinoids was determined using both foliar and systemic uptake assays. Field populations were tested for resistance to the registered insecticides acetamiprid and dinotefuran.

Imidacloprid

Results from the systemic bioassays conducted in 2020 indicate that most field-collected populations tested were resistant to imidacloprid (Table 4). As a discriminating dose has not been developed locally, this is based off survival at 30mg/L, which effectively controls the lab-susceptible strain; 16 mg/L has been used internationally (Cahill et al., 1996). Combined with the resistance ratios this indicates that populations from Emerald, Darling Downs, St George, Macintyre, North Star, Moree, and Namoi Valley have resistance to imidacloprid.

Table 4. Summary of imidacloprid systemic bioassay results 2020

Population	LC_{50} (mg/L)	(95% FL)	Slope (SE)	Mortality (%) at 30 mg/L	Resistance Ratio
Susceptible	0.27	(0.14-0.44)	1.49 (0.26)	100	-
GR15-1R*	20.59	(15.66-27.79)	1.52 (0.14)	62.2	76
Gumlu†	11.19	(7.37-17.57)	1.35 (0.17)	85.2	41
Bowen†	17.84	(9.72-39.39)	1.12 (0.19)	47.4	66
Emerald	8.32	(5.90-11.95)	1.32 (0.13)	67.4	31
Theodore	0.71	(0.49-1.02)	1.10 (0.13)	100	2.6
Darling Downs	13.93	(9.57-20.62)	1.97 (0.29)	84.0	51
St George (1)	2.56	(1.99-3.28)	1.85 (0.17)	98.0	9.4
St George (2)	3.95	(3.04-5.12)	1.42 (0.11)	98.1	15
Macintyre (1)	9.17	(6.28-13.87)	1.19 (0.12)	69.7	34
Macintyre (2)	7.46	(5.55-10.15)	1.33 (0.12)	83.0	27
North Star	12.57	(8.84-18.35)	1.38 (0.15)	59.3	46
Moree (1)	10.12	(8.37-12.24)	1.98 (0.15)	81.4	37
Moree (2)	4.17	(2.81-6.23)	1.41 (0.16)	89.9	15
Moree (3)	9.96	(7.07-14.27)	1.64 (0.19)	85.1	37
Namoi valley (1)	3.00	(1.89-4.87)	1.07 (0.13)	94.0	11
Namoi valley (2)	4.96	(3.89-6.39)	1.72 (0.14)	93.9	18
Namoi valley (3)	7.32	(6.10-8.80)	2.08 (0.16)	89.7	27

Population	LC_{50} (mg/L)	(95% FL)	Slope (SE)	Mortality (%) at 30 mg/L	Resistance Ratio
Narromine	2.05	(1.63-2.59)	1.74 (0.15)	100	7.5
Hillston	0.61	(0.32-1.07)	1.18 (0.20)	100	2.3

***** Neonicotinoid resistant strain

† Populations collected from horticultural crops

Pymetrozine

The foliar bioassay results from 2020 show most populations were resistant to pymetrozine (Table 5). In many cases the assays failed to reach 50% mortality which makes extrapolation of results difficult. At 100 mg/L, mortality >75% was only recorded in the St George (1), Namoi Valley (2), Narromine and Hillston populations. Except for the Theodore population, which was susceptible to imidacloprid, cross-resistance between pymetrozine and imidacloprid was observed.

Table 5. Summary of pymetrozine bioassay results 2020

Population	LC_{50} (mg/L)	(95% FL)	Slope (SE)	Mortality (%) at 100 mg/L	Resistance Ratio
Susceptible	4.1	(3.0-5.8)	1.89 (0.22)	100	-
Gumlu†	>100	-	0.24 (0.19)	7.8	>20
Bowen†	>100	-	-	3.1	>20
Emerald	>100	-	0.48 (0.16)	26.7	65
Theodore	166.7	(109.3-273.6)	0.89 (0.01)	38.5	40
Darling Downs	606	(303.5-1947)	0.87 (0.15)	15.8	147
St George (1)	46.7	(16.36-128.7)	0.80 (0.19)	80.4	11.3
St George (2)	67.4	(40.1-116.7)	0.83 (0.11)	59.0	16.4
Macintyre (1)	>100	-	0.60 (0.17)	23.8	>20
Macintyre (2)	>100	-	0.44 (0.13)	27.2	>20
North Star	>100	-	0.59 (0.18)	12.7	>20
Moree (1)	>100	-	0.68 (0.16)	20.5	>20
Moree (2)	>100	-	0.81 (0.29)	15.3	>20
Moree (3)	171.1	(93.8-553.7)	1.28 (0.24)	44.0	42
Namoi valley (1)	90.9	(56.1-155.0)	1.00 (0.13)	50.0	22
Namoi valley (2)	24.3	(9.31-50.2)	0.46 (0.09)	76.0	5.9
Namoi valley (3)	34.4	(14.09-72.7)	0.51 (0.10)	73.2	8.4
Narromine	10.1	(7.1-14.5)	1.67 (0.21)	100	2.5
Hillston	14.9	(10.5-21.6)	1.63 (0.20)	91.0	3.6

† Populations collected from horticultural crops

Lab resistant strain – GR15-1R

The GR15-1 R strain was tested with thiacloprid, imidacloprid, thiamethoxam, clothianidin, acetamiprid and dinotefuran using a foliar assay (Table 6). The results demonstrated that GR15-1R has high to very high resistance to thiacloprid, imidacloprid, thiamethoxam, and clothianidin. Except for thiamethoxam (79%), mortality in the above assays was <50% at 1000 mg/L. The acetamiprid assay did achieve 100% mortality at 1000 mg/L, while at 320 mg/L mortality was 98.9%. The resistance ratio was 11.4; suggesting low level cross-resistance to acetamiprid. In the dinotefuran assay 100% mortality was recorded at 320 mg/L, indicating there was no cross resistance to dinotefuran.

The systemic results showed a significant increase in the sensitivity of GR15-1R to neonicotinoids (Table 7). The bioassays showed resistance to imidacloprid and clothianidin, with neither assay reaching 100% mortality at 100 mg/L and resistance ratios were >10. The thiamethoxam assay indicated the presence of low level cross-resistance, while for the other neonicotinoids (thiacloprid, acetamiprid & dinotefuran) there was limited evidence of cross resistance.

The conclusion from this study is whitefly that are resistant to imidacloprid and clothianidin have low cross resistance to acetamiprid, but no cross resistance dinotefuran. Cross resistance to thiamethoxam is limited if applied systemically.

Table 6. Foliar bioassay, dose response of neonicotinoid resistant population (GR15-1R) and susceptible population (SU07-1) to neonicotinoids

Insecticide	Population	LC_{50} (mg/L) (95% FL)	Slope (SE)	Minimum Effective Concentration (mg/L)	Resistance Ratio
Thiacloprid	SU07-1	1.95 (1.0–3.2)	1.24 (0.21)	100	-
	GR15-1R	>1000	0.34 (0.12)	-	> 512
Imidacloprid	SU07-1	2.652 (1.7–3.9)	2.11 (0.37)	100	-
	GR15-1R	>1000	0.47 (0.11)	-	>377
Thiamethoxam	SU07-1	7.91 (5.4–11.2)	1.54 (0.18)	320	-
	GR15-1R	358.1 (234.2–623.6)	1.67 (0.28)	-	45.2
Clothianidin	SU07-1	22.57 (11.1–44.4)	1.01 (0.17)	320	-
	GR15-1R	>1000	0.40 (0.15)	-	> 44
Acetamiprid	SU07-1	1.192 (0.9–1.5)	2.34 (0.29)	32	
	GR15-1R	13.61 (10.3–17.9)	1.60 (0.14)	1000	11.4
Dinotefuran	SU07-1	2.82 (2.1–3.8)	2.61 (0.41)	32	
	GR15-1R	17.82 (13.3– 23.8)	2.34 (0.28)	320	6.3

Table 7. Systemic bioassay, dose response of neonicotinoid resistant population (GR15-1R) and susceptible population (SU07-1) to neonicotinoids

Insecticide	Population	LC_{50} (mg/L) (95% FL)	Slope (SE)	Minimum Effective Concentration (mg/L)	Resistance Ratio
Thiacloprid	SU07-1	1.73 (0.7–3.9)	1.09 (0.22)	100	-
	GR15-1R	4.92 (3.6 –6.9)	1.86 (0.21)	100	2.8
Imidacloprid	SU07-1	0.93 (0.6 –1.4)	1.41 (0.16)	100	-
	GR15-1R	21.19 (16.1 –28.6)	1.53 (0.15)	-	22.7
Thiamethoxam	SU07-1	0.87 (0.5 –1.4)	1.85 (0.32)	10	-
	GR15-1R	5.83 (4.6 –7.5)	2.62 (0.32)	100	6.7
Clothianidin	SU07-1	7.47 (5.1 –11.1)	1.94 (0.27)	100	-
	GR15-1R	88.46 (42.3 –498.1)	1.28 (0.34)	-	11.8
Acetamiprid	SU07-1	0.63 (0.4 –1.0)	1.82 (0.32)	10	-
	GR15-1R	2.46 (1.8 –3.4)	1.92 (0.22)	100	3.9
Dinotefuran	SU07-1	0.22 (0.2 –0.3)	3.89 (0.71)	1	-
	GR15-1R	1.07 (0.7 –1.6)	2.14 (0.36)	10	4.9

Dinotefuran

Due to its minor use, testing of dinotefuran was reduced, typically to a single population per cotton production region (Table 8). Populations tested were susceptible and bioassay results showed 100% mortality at 320 mg/L, well below the discriminating dose of 600 mg/L.

Table 8. Summary of dinotefuran bioassay results

Population	Year	LC_{50} (mg/L)	(95% FL)	Slope (SE)	Mortality (%) at 320 mg/L	Resistance Ratio
Susceptible	-	12.31	(8.34-18.22)	1.58 (0.19)	100	-
Emerald	2020	11.23	(7.15-16.48)	1.38 (0.16)	100	0.9
Theodore	2020	5.55	(3.61-8.02)	1.97 (0.26)	100	0.5
Darling Downs	2020	14.1	(8.84-20.22)	2.19 (0.39)	100	1.1
St George (1)	2020	7.12	(5.28-9.22)	2.36 (0.28)	100	0.6
North Star	2020	22.48	(19.49-25.91)	3.62 (0.37)	100	1.8
Moree (3)	2020	16.89	(12.33-22.1)	2.21 (0.30)	100	1.4
Namoi valley (2)	2020	5.76	(4.52-7.12)	2.57 (0.25)	100	0.5
Narromine	2020	9.56	-	-	100	0.7
Hillston	2020	6.77	(4.89-8.63)	3.02 (0.46)	100	0.5
Emerald	2021	10.16	(7.11-13.53)	2.35 (0.32)	100	0.8

Population	Year	LC_{50} (mg/L)	(95% FL)	Slope (SE)	Mortality (%) at 320 mg/L	Resistance Ratio
Theodore	2021	8.47	(6.76-10.38)	1.83 (0.14)	100	0.7
St George (1)	2021	13.46	(10.07-17.50)	1.29 (0.10)	100	1.1
Macintyre (3)	2021	13.72	(11.07-16.63)	1.99 (0.17)	100	1.1
Moree (1)	2021	12.51	(8.13-17.94)	1.74 (0.23)	100	1.0
Namoi valley (3)	2021	13.62	(10.48-17.28)	1.64 (0.14)	100	1.1
Narromine	2021	16.4	(10.92-23.29)	1.90 (0.26)	100	1.3
Hillston	2021	8.03	(5.42-11.08)	2.18 (0.30)	100	0.7
Griffith	2021	11.02	(8.86-13.51)	1.81 (0.14)	100	0.9
Theodore	2022	9.04	(7.29-10.92)	2.31 (0.21)	100	0.7
St George (1)	2022	17.10	(13.94-20.63)	1.98 (0.17)	100	1.4
Mungindi (1)	2022	42.91	(37.64-48.91)	4.03 (0.41)	320	3.5
Macintyre (2)	2022	25.05	(18.81-33.3)	3.02 (0.48)	100	2.0
Moree	2022	16.85	(11.17-23.93)	1.78 (0.24)	100	1.4
Namoi valley (3)	2022	16.31	(13.40-19.41)	3.19 (0.39)	320	1.3

Acetamiprid

Acetamiprid is registered for silverleaf whitefly control both on its own (since 2020), and as a co-formulation with emamectin benzoate. Bioassays of 45 populations of silverleaf whitefly tested their toxicity response to acetamiprid (Table 9). Over the three years of testing resistance ratios ranged between 0.6 and 4.2, and all populations recorded 100% mortality at doses ≤300 mg/L indicating the absence of clearly resistant populations. A minimum effective concentration of 300 mg/L likely represents the upper limit of field vigour tolerance.

Table 9. Summary of acetamiprid bioassay results

Population	Year	LC_{50} (mg/L)	(95% FL)	Slope (SE)	Min. Effective Conc. (mg/L)	Resistance Ratio
Susceptible	-	4.02	(3.13-5.21)	2.42 (0.28)	32	-
Emerald	2020	8.93	(6.29-12.42)	1.33 (0.14)	300	2.2
Theodore	2020	7.35	(5.40-9.97)	1.66 (0.17)	100	1.8
Darling Downs	2020	10.11	(7.55-13.47)	2.21 (0.26)	300	2.5
St George (1)	2020	3.52	(2.58-4.63)	1.57 (0.17)	100	0.9
St George (2)	2020	4.83	(3.81-6.14)	2.10 (0.23)	30	1.2
Macintyre (1)	2020	4.99	(3.56-6.77)	1.36 (0.15)	100	1.2
Macintyre (2)	2020	12.65	(9.77-16.4)	1.77 (0.17)	300	3.1
North Star	2020	5.55	(4.14-7.43)	2.40 (0.32)	30	1.4
Moree (1)	2020	6.03	(4.88-7.39)	1.69 (0.13)	100	1.5
Moree (2)	2020	11.69	(8.82-15.58)	1.84 (0.19)	100	2.9
Moree (3)	2020	5.72	(3.98-8.20)	2.08 (0.31)	30	1.4
Namoi valley (1)	2020	2.37	(1.59-3.33)	1.95 (0.30)	30	0.6
Namoi valley (2)	2020	2.38	(2.01-2.79)	2.32 (0.21)	30	0.6
Namoi valley (3)	2020	1.69	(1.15-2.26)	1.38 (0.16)	30	0.4
Narromine	2020	3.04	(2.34-3.92)	2.54 (0.33)	100	0.8
Hillston	2020	4.30	(3.46-5.35)	2.86 (0.33)	100	1.1
Emerald	2021	4.91	(3.75-6.28)	1.25 (0.10)	300	1.2
Theodore	2021	10.44	(8.54-12.78)	1.66 (0.12)	300	2.6
St George (1)	2021	16.03	(12.08-21.28)	1.90 (0.19)	300	4.0
St George (2)	2021	10.74	(7.83-14.6)	2.15 (0.27)	100	2.7
St George (3)	2021	6.06	(5.15-7.11)	2.57 (0.21)	100	1.5
Macintyre (2)	2021	7.60	(6.38-9.04)	1.89 (0.13)	300	1.9
Macintyre (3)	2021	11.2	(8.92-14.07)	1.32 (0.09)	300	2.8
Moree (1)	2021	7.46	(6.19-8.92)	1.61 (0.10)	300	1.9
Moree (2)	2021	6.69	(4.96-9.16)	1.78 (0.22)	30	1.7
Moree (3)	2021	5.75	(4.38-7.50)	2.01 (0.21)	100	1.4
Namoi valley (1)	2021	8.10	(6.55-9.95)	1.65 (0.12)	100	2.0
Namoi valley (2)	2021	9.26	(7.37-11.55)	1.71 (0.14)	300	2.3
Namoi valley (3)	2021	11.52	(9.27-14.26)	1.55 (0.11)	300	2.9
Narromine	2021	5.88	(4.76-7.20)	1.62 (0.12)	300	1.5
Hillston	2021	3.20	(2.60-3.88)	1.53 (0.11)	100	0.8
Griffith	2021	3.30	(2.52-4.21)	1.65 (0.16)	100	0.8
Theodore	2022	3.76	(3.17-4.45)	2.45 (0.21)	100	0.9
St George (1)	2022	5.96	(4.81-7.33)	1.48 (0.11)	300	1.5
St George (2)	2022	8.57	(6.60-11.09)	1.85 (0.18)	100	2.1

Population	Year	LC_{50} (mg/L)	(95% FL)	Slope (SE)	Min. Effective Conc. (mg/L)	Resistance Ratio
St George (3)	2022	12.27	(9.50-15.88)	1.80 (0.17)	300	3.1
Mungindi (1)	2022	14.57	(12.28-17.32)	2.36 (0.19)	100	3.6
Mungindi (2)	2022	10.76	(8.47-13.62)	1.82 (0.15)	300	2.7
Macintyre (1)	2022	11.28	(8.62-14.66)	1.55 (0.13)	300	2.8
Macintyre (2)	2022	11.49	(9.47-13.95)	1.65 (0.11)	300	2.9
Macintyre (3)	2022	14.41	(12.07-17.22)	1.90 (0.13)	300	3.6
Moree	2022	16.90	(14.05-20.33)	1.85 (0.13)	300	4.2
Namoi valley (1)	2022	7.66	(5.81-10.14)	1.92 (0.21)	100	1.9
Namoi valley (2)	2022	9.65	(8.30-11.22)	2.66 (0.21)	300	2.4
Namoi valley (3)	2022	9.08	(7.62-10.80)	2.14 (0.16)	300	2.3

4D – Butenolides

Flupyradifurone

Flupyradifurone (Sivanto® prime), is a newly registered insecticide for silverleaf whitefly control on horticultural crops (e.g., cucurbits, tomatoes, and green beans). It cannot be applied by aircraft and to date has not been registered for use in cotton. However, given it's a new mode of action in Australia it was considered prudent to determine the baseline susceptibility of silverleaf whitefly to flupyradifurone to make it easier to detect resistance should it evolve, and further testing be required.

Both foliar and systemic uptake bioassays were evaluated, with both performing adequately. As expected, the systemic assay had greater sensitivity, and 100% mortality was achieved in each assay (Table 10). In the foliar assay, three populations, Emerald, Macintyre (2), and Griffith did not reach 100% mortality at the highest dose of 100 mg/L, with mortalities of 95.8, 97.0 and 99.0% respectively (Table 11). The LC_{99} of the lab susceptible population was 54.6, while for field-collected populations the average $LC_{99}$was 256.87. This suggests the addition of 300 mg/L is required to complete the dosage range for the foliar assay. Due to the limited testing and variability in response a discriminating dose was not determined for the foliar assay.

In the systemic assay, the LC_{99} of the susceptible was 2.30 and the $LC_{99.9}$ was 5.21. The average response of the field collected populations were an LC_{99} of 4.9 and an $LC_{99.9}$ of 12.3. The Griffith population had the highest recorded $LC_{99.9}$ at 23.99. A preliminary discriminating dose of 30 mg/L for the systemic assay was determined from the data.

Table 10. Summary of flupyradifurone systemic uptake bioassay results

Population	LC_{50} (mg/L)	(95% FL)	Slope (SE)	Min. Effective Conc. (mg/L)	Resistance Ratio
Susceptible	0.192	(0.139-0.266)	2.15 (0.28)	3	-
Emerald	0.431	(0.360-0.519)	3.62 (0.44)	3	2.3
Theodore	0.297	(0.197-0.445)	1.69 (0.23)	3	1.5
St George (1)	0.352	(0.207-0.543)	1.41 (0.22)	3	1.8
Macintyre (2)	0.467	(0.359-0.601)	2.42 (0.30)	10	2.4
Moree (1)	0.177	(0.114-0.248)	2.31 (0.42)	3	0.9
Namoi valley (3)	0.655	(0.360-1.119)	1.76 (0.34)	10	3.4
Narromine	0.335	(0.243-0.456)	2.47 (0.35)	3	1.8
Hillston	0.260	(0.206-0.331)	3.22 (0.45)	3	1.4
Griffith	0.344	(0.222-0.505)	1.68 (0.26)	10	1.8

Table 11. Summary of flupyradifurone foliar bioassay results

Population	LC_{50} (mg/L)	(95% FL)	Slope (SE)	Min. Effective Conc. (mg/L)	Resistance Ratio
Susceptible	1.79	(0.95-2.74)	1.57 (0.28)	100	-
Emerald	14.00	(10.85-18.15)	2.02 (0.21)	-	7.8
Theodore	5.61	(3.98-7.83)	1.95 (0.26)	100	3.1
St George (1)	12.20	(10.03-14.88)	1.58 (0.12)	100	6.8
Macintyre (2)	22.52	(16.79-31.25)	1.69 (0.19)	-	12.6
Moree (1)	14.31	(10.89-18.94)	1.89 (0.20)	100	8.0

Population	LC_{50} (mg/L)	(95% FL)	Slope (SE)	Min. Effective Conc. (mg/L)	Resistance Ratio
Namoi valley (3)	14.57	(12.18-17.49)	1.82 (0.13)	100	8.2
Narromine	8.48	(6.25-11.49)	1.58 (0.17)	100	4.8
Hillston	9.56	(7.29-12.62)	1.67 (0.16)	100	5.4
Griffith	12.95	(11.07-15.15)	2.44 (0.19)	-	7.3

6 – Avermectins

Emamectin Benzoate

Between 2020 and 2022, 45 populations were tested for resistance to emamectin benzoate (Table 12). Emamectin benzoate has been included in testing as it registered as a co-formulation with acetamiprid. The minimum effective concentration required to achieve 100% mortality in the field collected populations was ≤10 mg/L (equivalent to the lab susceptible population). The field-collected populations had low resistance ratios of ≤3.6. From these results it can be concluded none of the tested populations had resistance to emamectin benzoate.

Table 12. Summary of emamectin benzoate bioassay results

Population	Year	LC_{50} (mg/L)	(95% FL)	Slope (SE)	Mortality (%) at 10 mg/L	Resistance Ratio
Susceptible	-	0.90	(0.72-1.12)	2.74 (0.31)	100	-
Emerald	2020	1.23	(0.76-2.06)	1.65 (0.27)	100	1.4
Theodore	2020	1.16	(0.81-1.70)	2.2 (0.32)	100	1.3
Darling Downs	2020	1.64	(1.42-1.89)	3.2 (0.31)	100	1.8
St George (1)	2020	0.99	(0.82-1.19)	3.13 (0.36)	100	1.1
St George (2)	2020	2.54	(1.94-3.36)	3.67 (0.67)	100	2.8
Macintyre (1)	2020	0.80	(0.61-1.04)	2.23 (0.25)	100	0.9
Macintyre (2)	2020	1.77	(1.28-2.48)	2.01 (0.27)	100	2.0
North Star	2020	0.92	(0.67-1.3)	2.97 (0.48)	100	1.0
Moree (1)	2020	1.13	(0.78-1.64)	1.81 (0.25)	100	1.2
Moree (2)	2020	2.65	(2.09-3.40)	3.30 (0.46)	100	2.9
Moree (3)	2020	0.99	(0.74-1.33)	2.57 (0.34)	100	1.1
Namoi valley (1)	2020	1.32	(1.03-1.70)	2.40 (0.27)	100	1.5
Namoi valley (2)	2020	0.95	(0.84-1.09)	2.74 (0.20)	100	1.1
Namoi valley (3)	2020	1.36	(1.03-1.80)	2.17 (0.26)	100	1.5
Narromine	2020	1.60	(1.25-2.05)	2.31 (0.24)	100	1.8
Hillston	2020	1.95	(1.51-2.53)	3.03 (0.44)	100	2.2
Emerald	2021	0.61	(0.52-0.72)	2.52 (0.21)	100	0.7
Theodore	2021	1.23	(1.04-1.45)	3.51 (0.40)	100	1.4
St George (1)	2021	2.15	(1.68-2.73)	2.80 (0.37)	100	2.4
St George (2)	2021	1.76	(1.27-2.43)	2.67 (0.42)	100	1.9
St George (3)	2021	1.18	(0.93-1.49)	2.84 (0.34)	100	1.3
Macintyre (2)	2021	1.11	(0.92-1.34)	2.92 (0.29)	100	1.2
Macintyre (3)	2021	0.90	(0.65-1.24)	1.92 (0.23)	100	1.0
Moree (1)	2021	0.96	(0.83-1.11)	2.86 (0.24)	100	1.1
Moree (2)	2021	1.33	(1.07-1.63)	2.70 (0.28)	100	1.5
Moree (3)	2021	1.41	(1.13-1.75)	2.86 (0.33)	100	1.6
Namoi valley (1)	2021	1.14	(0.97-1.35)	3.14 (0.31)	100	1.3
Namoi valley (2)	2021	1.64	(1.32-2.02)	3.41 (0.46)	100	1.8
Namoi valley (3)	2021	1.42	(1.15-1.74)	2.87 (0.31)	100	1.6
Narromine	2021	0.76	(0.53-1.09)	2.47 (0.38)	100	0.8
Hillston	2021	1.52	(1.22-1.88)	3.37 (0.46)	100	1.7
Griffith	2021	1.13	(0.82-1.56)	1.92 (0.23)	100	1.3
Theodore	2022	1.10	(0.87-1.39)	2.85 (0.35)	100	1.2
St George (1)	2022	1.03	(0.73-1.43)	3.11 (0.57)	100	1.1
St George (2)	2022	1.00	(0.69-1.44)	1.94 (0.26)	100	1.1
St George (3)	2022	1.89	(1.63-2.18)	3.07 (0.28)	100	2.1
Mungindi (1)	2022	1.87	(1.13-3.34)	1.49 (0.26)	100	2.1
Mungindi (2)	2022	2.33	(1.95-2.79)	3.61 (0.45)	100	2.6
Macintyre (1)	2022	3.17	(2.46-4.11)	3.27 (0.48)	100	3.5
Macintyre (2)	2022	3.25	(2.85-3.70)	3.90 (0.38)	100	3.6
Macintyre (3)	2022	3.11	(2.43-4.00)	3.03 (0.42)	100	3.4
Moree	2022	2.50	(2.18-2.86)	3.88 (0.40)	100	2.8
Namoi valley (1)	2022	2.03	(1.75-2.36)	2.90 (0.26)	100	2.2
Namoi valley (2)	2022	2.63	(2.02-3.45)	2.78 (0.40)	100	2.9

Population	Year	LC_{50} (mg/L)	(95% FL)	Slope (SE)	Mortality (%) at 10 mg/L	Resistance Ratio
Namoi valley (3)	2022	2.43	(1.88-3.17)	2.95 (0.43)	100	2.7

7C – Pyriproxyfen

Using a discriminating dose of 10 mg/L, resistance to pyriproxyfen was detected in 2 populations in 2020, 9 populations in 2021 and 6 populations in 2022 (Table 13). To describe the level of resistance in a population, the resistance ratio is used. In 2020, Macintyre (2) & Moree (1) had moderate resistance. In 2021, 3 populations (Macintyre (2), Macintyre (3) & Namoi Valley (3)) had low resistance, 4 populations (Moree (2), Moree (3), Namoi Valley (1) & Namoi Valley (2)) had moderate resistance, and two populations (Moree (1) and Hillston) had high resistance. In 2022, Mungindi (2) had low resistance, while five populations (St George (1) and (3), Moree (3), Namoi Valley (1) and (3)) had moderate resistance.

Table 13. Summary of pyriproxyfen bioassay results

Population	Year	LC_{50} (mg/L)	(95% FL)	Slope (SE)	Mortality (%) at discriminating dose (10 mg/L)	Resistance Ratio
Susceptible	-	0.018	(0.014-0.024)	2.64 (0.34)	100	-
Emerald	2020	0.018	(0.011-0.028)	1.03 (0.10)	100	1
Theodore	2020	0.003	(0.001-0.008)	1.02 (0.19)	100	0.2
Darling Downs	2020	0.05	(0.035-0.076)	1.51 (0.14)	100	2.8
St George (1)	2020	0.09	(0.065-0.125)	1.08 (0.06)	100	4.9
St George (2)	2020	0.03	(0.017-0.043)	1.20 (0.12)	100	1.5
St George (3)	2020	0.09	(0.065-0.128)	1.47 (0.12)	100	5
Macintyre (1)	2020	0.16	(0.105-0.255)	1.40 (0.14)	100	9
Macintyre (2)	2020	0.26	(0.164-0.374)	1.32 (0.12)	99.2	13.7
North Star	2020	0.16	(0.108-0.230)	1.31 (0.11)	100	8.7
Moree (1)	2020	0.27	(0.208-0.337)	1.46 (0.09)	99.3	14.4
Moree (2)	2020	0.42	(0.258-0.643)	1.53 (0.18)	100	22.9
Moree (3)	2020	0.24	(0.187-0.294)	1.30 (0.07)	100	12.7
Namoi valley (1)	2020	0.2	(0.156-0.257)	1.15 (0.06)	100	10.9
Namoi valley (2)	2020	0.14	(0.101-0.192)	1.48 (0.11)	100	7.6
Namoi valley (3)	2020	0.25	(0.196-0.310)	1.50 (0.08)	100	13.4
Narromine	2020	0.06	(0.039-0.083)	1.59 (0.18)	100	3.1
Hillston	2020	0.21	(0.087-0.413)	1.21 (0.19)	100	11.1
Emerald	2021	0.024	(0.015-0.039)	1.34 (0.16)	100	1.3
Theodore	2021	0.073	(0.050-0.105)	1.42 (0.13)	100	3.9
St George (1)	2021	0.180	(0.142-0.224)	1.45 (0.08)	100	9.7
St George (2)	2021	0.173	(0.122-0.237)	1.51 (0.12)	100	9.3
St George (3)	2021	0.218	(0.176-0.267)	1.50 (0.08)	100	11.7
Macintyre (2)	2021	0.199	(0.136-0.278)	1.42 (0.12)	99.6	10.7
Macintyre (3)	2021	0.097	(0.075-0.124)	1.04 (0.05)	98.4	5.2
Macintyre (4)	2021	0.065	(0.042-0.098)	1.13 (0.09)	100	3.5
Moree (1)	2021	1.048	(0.738-1.391)	1.90 (0.22)	97.2	56.4
Moree (2)	2021	0.461	(0.376-0.557)	1.51 (0.08)	97.5	24.8
Moree (3)	2021	0.565	(0.355-0.831)	1.42 (0.16)	99.2	30.4
Namoi valley (1)	2021	0.219	(0.134-0.334)	1.09 (0.10)	97.2	11.8
Namoi valley (2)	2021	0.278	(0.154-0.463)	0.94 (0.10)	99.5	15.0
Namoi valley (3)	2021	0.185	(0.129-0.258)	1.13 (0.08)	99.2	10.0
Narromine	2021	0.358	(0.257-0.478)	1.72 (0.15)	100	19.3
Hillston	2021	0.671	(0.367-1.100)	1.23 (0.16)	99.2	36.1
Griffith	2021	0.073	(0.046-0.111)	0.97 (0.07)	100	3.9
Theodore	2022	0.033	(0.018-0.055)	0.81 (0.07)	100	1.8
St George (1)	2022	0.323	(0.251-0.408)	1.14 (0.06)	99.6	17.4
St George (2)	2022	0.144	(0.084-0.232)	1.16 (0.12)	100	7.8
St George (3)	2022	0.206	(0.128-0.313)	1.09 (0.09)	99.6	11.1
Mungindi (1)	2022	0.335	(0.239-0.451)	1.44 (0.11)	100	18.0
Mungindi (2)	2022	0.173	(0.089-0.306)	1.13 (0.13)	99.6	9.3
Macintyre (1)	2022	0.122	(0.094-0.155)	1.25 (0.07)	100	6.6
Macintyre (2)	2022	0.086	(0.066-0.109)	1.18 (0.06)	100	4.6
Macintyre (3)	2022	0.158	(0.123-0.199)	1.26 (0.07)	100	8.5

Population	Year	LC_{50} (mg/L)	(95% FL)	Slope (SE)	Mortality (%) at discriminating dose (10 mg/L)	Resistance Ratio
Moree	2022	0.501	(0.299-0.777)	1.10 (0.12)	99.2	26.9
Namoi valley (1)	2022	0.275	(0.220-0.339)	1.36 (0.07)	99.6	14.8
Namoi valley (2)	2022	0.391	(0.271-0.536)	1.60 (0.14)	100	21.0
Namoi valley (3)	2022	0.502	(0.368-0.661)	1.31 (0.10)	99.6	27.0

9D – Pyropenes

Afidopyropen

Baseline susceptibility of afidopyropen (Versys®) was tested in 2020 (Table 14). Afidopyropen is registered in cotton for suppression of silverleaf whitefly. Application of afidopyropen is restricted; it cannot be applied by aircraft, limiting its adoption for use against whitefly in cotton.

A foliar assay was used to evaluate the susceptibility of field-collected populations to afidopyropen. The tested populations had decreased sensitivity compared to the lab susceptible strain, but not significant enough (RR = 0.9 to 5.9, MEC ≤100 mg/L) to suggest existing resistance to this mode of action. The dose response of the 2020 populations, particularly from Bowen would indicate no apparent cross-resistance to pymetrozine (9B), a similar finding to Zhang et al. (2021).

Dose responses observed in afidopyropen bioassays were often highly variable between replicates, and in some assays 100% mortality was not achieved within the dose range, which meant in some cases assays needed to be repeated. This observation is likely linked to the mode of action of group 9 insecticide (chordotonal organ TRPV channel modulators) which indirectly kills sucking pests by inhibiting feeding, leading to starvation and eventual death (Zhang et al., 2021).

A discriminating dose of 300 mg/L was determined from the dose response of 37 populations tested between 2019 and 2020. It was set using the parameters of highest LC_{99} value recorded, 229.3 (104.6-880.8) and the highest recorded minimum effective concentration of 100 mg/L.

Table 14. Summary of afidopyropen bioassay results 2020

Population	LC_{50} (mg/L)	(95% FL)	Slope (SE)	Min. Effective Conc. (mg/L)	Resistance Ratio
Susceptible	2.76	(1.92-4.07)	2.50 (0.44)	10	-
Gumlu	4.68	(3.64-6.11)	2.69 (0.39)	30	1.7
Bowen	7.41	(4.89- 11.85)	1.81 (0.33)	100	2.7
Emerald	6.66	(5.00-9.41)	2.66 (0.45)	30	2.4
Theodore	5.27	(3.73-7.2)	2.25 (0.40)	100	1.9
Darling Downs	3.11	(1.33-5.73)	1.49 (0.39)	100	1.1
St George (1)	14.11	(9.78-21.91)	1.98 (0.34)	100	5.1
St George (2)	5.66	(4.09-8.08)	2.11 (0.33)	100	2.6
Macintyre (1)	6.39	(4.74-8.94)	2.11 (0.31)	100	2.3
Macintyre (2)	11.56	(8.28-17.13)	1.79 (0.25)	100	4.2
North Star	9.50	(7.48-12.54)	2.40 (0.29)	100	3.4
Moree (1)	8.84	(6.43-12.95)	2.49 (0.41)	100	3.2
Moree (2)	7.70	(5.32-11.72)	1.87 (0.30)	100	2.8
Moree (3)	9.86	(7.63-13.22)	1.93 (0.22)	100	3.6
Namoi valley (1)	2.57	(1.64-3.84)	2.94 (0.74)	10	0.9
Namoi valley (2)	5.75	(4.11-8.47)	2.77 (0.54)	30	2.1
Namoi valley (3)	3.47	(2.11-5.59)	1.99 (0.46)	30	1.3
Narromine	9.15	(7.24-11.95)	2.32 (0.27)	100	3.3
Hillston	3.60	(2.52-5.05)	1.83 (0.29)	100	1.3

12A – Diafenthiuron

For the duration of this project, technical grade diafenthiuron was used which resulted in lower LC_{50} response compared to those previously observed when formulated insecticide was used. All

populations reached 100% mortality at dose ≤ 30 mg/L and resistance ratios ranged between 0.4 and 1.9 (Tables 15). Resistance was not detected in any of the tested populations.

Table 15. Summary of diafenthiuron bioassay results

Population	Year	LC_{50} (mg/L)	(95% FL)	Slope (SE)	Mortality (%) at 30 mg/L	Resistance Ratio
Susceptible	-	3.32	(2.95-3.75)	3.89 (0.35)	100	-
Emerald	2020	3.89	(2.75-5.56)	2.11 (0.29)	100	1.7
Theodore	2020	2.61	(2.02-3.41)	3.37 (0.49)	100	0.8
Darling Downs	2020	2.07	(1.78-2.43)	3.40 (0.36)	100	0.6
St George (1)	2020	2.33	(2.01-2.71)	3.24 (0.32)	100	0.7
St George (2)	2020	1.79	(1.34-2.40)	3.46 (0.63)	100	0.5
St George (3)	2020	3.57	(2.60-4.93)	2.54 (0.37)	100	1.1
Macintyre (1)	2020	3.82	(2.88-5.07)	2.93 (0.43)	100	1.2
Macintyre (2)	2020	4.85	(3.43-7.07)	3.61 (0.79)	100	1.5
North Star	2020	6.26	(4.99-7.82)	2.93 (0.35)	100	1.9
Moree (1)	2020	2.19	(1.66-2.87)	3.23 (0.53)	100	0.7
Moree (2)	2020	2.69	(2.35-3.09)	3.68 (0.37)	100	0.8
Moree (3)	2020	2.79	(2.11-3.78)	2.49 (0.34)	100	0.8
Namoi valley (1)	2020	4.32	(3.37-5.66)	3.13 (0.48)	100	1.3
Namoi valley (2)	2020	5.00	(4.23-5.89)	2.94 (0.27)	100	1.5
Namoi valley (3)	2020	3.78	(3.03-4.73)	2.59 (0.28)	100	1.1
Narromine	2020	2.58	(2.27-2.94)	4.12 (0.44)	100	0.8
Hillston	2020	6.02	(4.85-7.36)	5.18 (0.83)	100	1.8
Emerald	2021	2.16	(1.73-2.73)	4.52 (0.80)	100	0.6
Theodore	2021	2.60	(1.75-4.14)	2.31 (0.45)	100	0.8
St George (1)	2021	2.94	(2.33-3.76)	4.01 (0.65)	100	0.9
St George (2)	2021	2.63	(2.15-3.25)	5.29 (1.08)	100	0.8
St George (3)	2021	1.32	(1.02-1.69)	4.55 (0.91)	100	0.4
Macintyre (2)	2021	2.51	(1.95-3.21)	2.41 (0.27)	100	0.8
Macintyre (3)	2021	2.65	(1.86-3.81)	2.66 (0.46)	100	0.8
Moree (1)	2021	3.25	(2.53-4.22)	3.08 (0.47)	100	1.0
Moree (2)	2021	3.09	(2.27-4.28)	2.97 (0.48)	100	0.9
Moree (3)	2021	4.59	(3.99-5.25)	4.12 (0.41)	100	1.4
Namoi valley (1)	2021	4.42	(3.84-5.10)	3.14 (0.27)	100	1.3
Namoi valley (2)	2021	3.76	(2.99-4.72)	2.85 (0.33)	100	1.1
Namoi valley (3)	2021	3.45	(2.86-4.16)	3.04 (0.31)	100	1.0
Narromine	2021	2.10	(1.42-3.15)	2.45 (0.46)	100	0.6
Hillston	2021	3.43	(3.02-3.92)	3.83 (0.38)	100	1.0
Griffith	2021	2.35	(1.52-3.74)	2.05 (0.39)	100	0.7
Theodore	2022	1.62	(1.20-2.18)	2.45 (0.35)	100	0.5
St George (1)	2022	1.82	(1.48-2.23)	5.22 (0.86)	100	0.5
St George (2)	2022	2.27	(1.99-2.60)	4.51 (0.51)	100	0.7
St George (3)	2022	1.16	(0.73-1.75)	2.57 (0.51)	100	0.3
Mungindi (1)	2022	1.17	(1.03-1.31)	4.08 (0.40)	100	0.4
Mungindi (2)	2022	1.37	(1.02-1.83)	2.19 (0.29)	100	0.4
Macintyre (1)	2022	2.28	(1.71-3.00)	3.71 (0.69)	100	0.7
Macintyre (2)	2022	2.41	(1.83-3.17)	3.47 (0.59)	100	0.7
Macintyre (3)	2022	3.75	(3.00-4.69)	3.34 (0.45)	100	1.1
Moree	2022	4.45	(3.36-5.95)	3.11 (0.49)	100	1.3
Namoi valley (1)	2022	2.85	(2.21-3.71)	3.40 (0.51)	100	0.9
Namoi valley (2)	2022	3.19	(2.59-3.95)	3.75 (0.53)	100	1.0
Namoi valley (3)	2022	2.99	(2.27-3.98)	3.08 (0.49)	100	0.9

16 – Buprofezin

The baseline susceptibility of 46 populations to buprofezin was determined and used for ongoing resistance screening. At LC_{50} field-collected populations showed limited deviation from the lab susceptible strain with resistance ratios between 0.4 and 3.1 (Table 16). At 100 mg/L mortality of most populations was 100%; exceptions were in 2020 North Star at 98%, and in 2022 Theodore 98.6%, Macintyre (3) at 99.1% and Namoi valley (3) at 99.3%. Based on several years of data a discriminating dose of 200 mg/L has been developed and will be used to distinguish between susceptible and resistant populations.

Table 16. Summary of buprofezin bioassay results

Population	Year	LC_{50} (mg/L)	(95% FL)	Slope (SE)	Mortality (%) at 100 mg/L	Resistance Ratio
Susceptible	-	1.11	(0.91-1.35)	2.37 (0.21)	100	-
Emerald	2020	1.35	(0.78-1.96)	1.18 (0.16)	100	1.2
Theodore	2020	3.26	(2.71-3.87)	1.72 (0.13)	100	2.9
Darling Downs	2020	1.47	(1.09-2.01)	1.52 (0.15)	100	1.3
St George (1)	2020	0.92	(0.59-1.24)	1.38 (0.20)	100	0.8
St George (2)	2020	1.60	(1.12-2.10)	2.12 (0.32)	100	1.4
St George (3)	2020	1.64	(1.30-1.98)	2.02 (0.20)	100	1.5
Macintyre (1)	2020	2.14	(1.34-2.98)	1.92 (0.29)	100	1.9
Macintyre (2)	2020	3.49	(2.79-4.32)	1.62 (0.16)	100	3.1
North Star	2020	2.45	(1.43-3.62)	1.14 (0.15)	98.0	2.2
Moree (1)	2020	1.06	(0.84-1.33)	2.17 (0.21)	100	1.0
Moree (2)	2020	0.95	(0.68-1.30)	2.35 (0.37)	100	0.9
Moree (3)	2020	0.91	(0.36-1.40)	1.61 (0.36)	100	0.8
Namoi valley (1)	2020	2.04	(1.52-2.60)	1.88 (0.22)	100	1.8
Namoi valley (2)	2020	1.82	(1.29-2.41)	1.64 (0.19)	100	1.6
Namoi valley (3)	2020	1.90	(1.23-2.64)	1.93 (0.30)	100	1.7
Narromine	2020	0.61	(0.51-0.71)	2.03 (0.19)	100	0.5
Hillston	2020	1.12	(0.80-1.56)	1.15 (0.11)	100	1.0
Emerald	2021	1.10	(0.81-1.46)	1.14 (0.09)	100	0.9
Theodore	2021	1.02	(0.72-1.43)	1.14 (0.11)	100	0.9
St George (1)	2021	0.7	(0.49-0.99)	1.78 (0.23)	100	0.6
St George (2)	2021	0.78	(0.56-1.08)	1.46 (0.16)	100	0.7
St George (3)	2021	0.72	(0.49-1.03)	1.40 (0.17)	100	0.6
Macintyre (2)	2021	0.43	(0.26-0.65)	1.45 (0.21)	100	0.4
Macintyre (3)	2021	0.59	(0.44-0.77)	2.21 (0.25)	100	0.5
Moree (1)	2021	0.59	(0.35-0.91)	1.10 (0.15)	100	0.5
Moree (2)	2021	0.58	(0.46-0.74)	2.63 (0.32)	100	0.5
Moree (3)	2021	0.54	(0.33-0.82)	1.21 (0.15)	100	0.5
Namoi valley (1)	2021	1.03	(0.82-1.32)	1.90 (0.18)	100	0.9
Namoi valley (2)	2021	1.26	(0.95-1.67)	1.93 (0.20)	100	1.1
Namoi valley (3)	2021	1.05	(0.84-1.32)	1.80 (0.20)	100	0.8
Narromine	2021	0.80	(0.53-1.15)	1.04 (0.10)	100	0.7
Hillston	2021	0.75	(0.56-1.00)	1.60 (0.17)	100	0.6
Griffith	2021	0.65	(0.49-0.87)	2.38 (0.32)	100	0.6
Theodore	2022	0.75	(0.60-0.93)	1.07 (0.07)	98.6	0.6
St George (1)	2022	1.49	(1.19-1.85)	1.98 (0.18)	100	1.3
St George (2)	2022	1.30	(0.91-1.86)	0.98 (0.09)	100	1.1
St George (3)	2022	1.60	(1.41-1.82)	2.35 (0.15)	100	1.4
Mungindi (1)	2022	0.90	(0.72-1.10)	1.38 (0.09)	100	0.8
Mungindi (2)	2022	0.67	(0.47-0.91)	1.99 (0.25)	100	0.6
Macintyre (1)	2022	1.11	(0.84-1.46)	1.34 (0.11)	100	1.0
Macintyre (2)	2022	1.00	(0.77-1.27)	1.30 (0.11)	100	1.2
Macintyre (3)	2022	1.50	(1.10-1.92)	1.30 (0.11)	99.1	1.2
Moree	2022	0.86	(0.74-0.99)	2.00 (0.13)	100	0.7
Namoi valley (1)	2022	0.70	(0.48-0.98)	1.42 (0.17)	100	0.6
Namoi valley (2)	2022	0.93	(0.72-1.20)	1.59 (0.14)	100	0.8
Namoi valley (3)	2022	1.08	(0.79-1.46)	1.41 (0.13)	99.3	0.9

23 – Tetronic and Tetramic acid derivatives

Spirotetramat

Minor to low level resistance to spirotetramat was detected in 6 out of 46 populations tested (Table 17). In 2020 two populations, Emerald, and Darling Downs (Dalby) survived the discriminating dose of 100 mg/L. In 2021, one population from Namoi Valley (Narrabri) was resistant and in 2022, populations from Theodore, Mungindi (1) and Macintyre (2) had survivors.

Sequencing of 85 populations collected between 2017 and 2021 detected the target site mutation *A2083V* in only 3 populations collected from cotton, Emerald (2019) at a frequency of 1.4%, Emerald (2020) at 1.2%, and Narrabri (2021) at 4.2%. The mutation was not detected in the Darling Downs field population. Sequencing of the populations mentioned above after they had

undergone selection in the glasshouse with spirotetramat at 1000-3000 mg/L showed a significant increase in the frequency of the *A2083V* mutation (Figure 1).

Table 17. Summary of spirotetramat bioassay results

Population	Year	LC_{50} (mg/L)	(95% FL)	Slope (SE)	Mortality (%) at discriminating dose (100 mg/L)	Resistance Ratio
Susceptible	-	4.08	(3.32-5.00)	2.62 (0.27)	100	-
Emerald	2020	7.89	(5.52-11.11)	1.28 (0.14)	98.7	1.9
Theodore	2020	5.22	(4.25-6.41)	2.47 (0.23)	100	1.3
Darling Downs	2020	7.04	(5.64-8.78)	2.05 (0.18)	98.7	1.7
St George (1)	2020	3.08	(2.34-3.97)	2.16 (0.25)	100	0.8
St George (2)	2020	4.77	(3.60-6.47)	2.83 (0.42)	100	1.2
St George (3)	2020	3.86	(2.98-4.98)	2.19 (0.25)	100	0.9
Macintyre (1)	2020	6.02	(4.69-7.73)	2.10 (0.23)	100	1.5
Macintyre (2)	2020	7.01	(4.92-9.70)	1.55 (0.18)	100	1.7
North Star	2020	4.70	(3.66-6.03)	2.12 (0.23)	100	1.2
Moree (1)	2020	3.70	(2.43-5.29)	1.07 (0.12)	100	0.9
Moree (2)	2020	4.65	(3.18-6.46)	1.34 (0.15)	100	1.1
Moree (3)	2020	3.68	(2.17-5.70)	1.49 (0.26)	100	0.9
Namoi valley (1)	2020	5.91	(4.32-8.05)	1.91 (0.22)	100	1.4
Namoi valley (2)	2020	4.83	(3.04-7.30)	1.35 (0.19)	100	1.2
Namoi valley (3)	2020	8.14	(5.90-11.17)	1.70 (0.19)	100	2.0
Narromine	2020	4.36	(3.34-5.68)	2.31 (0.28)	100	1.1
Hillston	2020	4.43	(3.83-5.11)	1.97 (0.13)	100	1.1
Emerald	2021	10.87	(8.54-13.97)	1.86 (0.17)	100	2.7
Theodore	2021	6.09	(4.86-7.57)	1.83 (0.16)	100	1.5
St George (1)	2021	3.59	(2.42-5.05)	1.55 (0.19)	100	0.9
St George (2)	2021	3.36	(2.19-4.77)	1.70 (0.24)	100	0.8
St George (3)	2021	2.98	(2.37-3.67)	1.96 (0.17)	100	0.7
Macintyre (2)	2021	8.83	(5.92-13.06)	1.52 (0.20)	100	2.2
Macintyre (3)	2021	6.61	(4.82-8.96)	1.49 (0.15)	100	1.6
Moree (1)	2021	1.23	(0.55-2.00)	1.48 (0.26)	100	0.3
Moree (2)	2021	2.26	(1.65-2.93)	1.94 (0.23)	100	0.6
Moree (3)	2021	2.35	(2.01-2.72)	2.46 (0.22)	100	0.6
Namoi valley (1)	2021	7.75	(6.30-9.64)	2.13 (0.21)	100	1.9
Namoi valley (2)	2021	3.23	(2.36-4.35)	2.33 (0.33)	100	0.8
Namoi valley (3)	2021	5.71	(3.59-8.65)	1.43 (0.21)	93	1.4
Narromine	2021	5.72	(4.26-7.60)	1.53 (0.15)	100	1.4
Hillston	2021	1.95	(1.11-2.91)	1.27 (0.16)	100	0.5
Griffith	2021	6.03	(4.59-7.92)	1.92 (0.20)	100	1.5
Theodore	2022	6.47	(4.59-9.00)	1.46 (0.17)	95.6	1.6
Theodore (selected)#	2022	157.9	(93.2-306.2)	0.68 (0.08)	42.2	38.7
St George (1)	2022	5.43	(3.47-7.95)	1.32 (0.18)	100	1.3
St George (2)	2022	4.86	(3.17-7.10)	1.16 (0.15)	100	1.2
St George (3)	2022	3.55	(2.87-4.30)	1.38 (0.10)	100	0.9
Mungindi (1)	2022	3.57	(2.38-5.07)	1.73 (0.24)	98.0	0.9
Mungindi (2)	2022	2.59	(1.99-3.26)	1.77 (0.18)	100	0.6
Macintyre (1)	2022	3.95	(2.84-5.35)	1.84 (0.22)	100	1.0
Macintyre (2)	2022	4.70	(2.92-6.99)	0.89 (0.11)	97.3	1.2
Macintyre (3)	2022	5.89	(4.18-8.13)	1.78 (0.21)	100	1.4
Moree	2022	4.20	(3.27-5.37)	2.69 (0.33)	100	1.0
Namoi valley (1)	2022	3.72	(2.81-4.91)	2.57 (0.35)	100	0.9
Namoi valley (2)	2022	4.22	(3.17-5.59)	2.40 (0.31)	100	1.0
Namoi valley (3)	2022	5.07	(4.16-6.17)	3.07 (0.34)	100	1.2

bioassay result of population following selection of earlier generation with 1000 mg/L spirotetramat

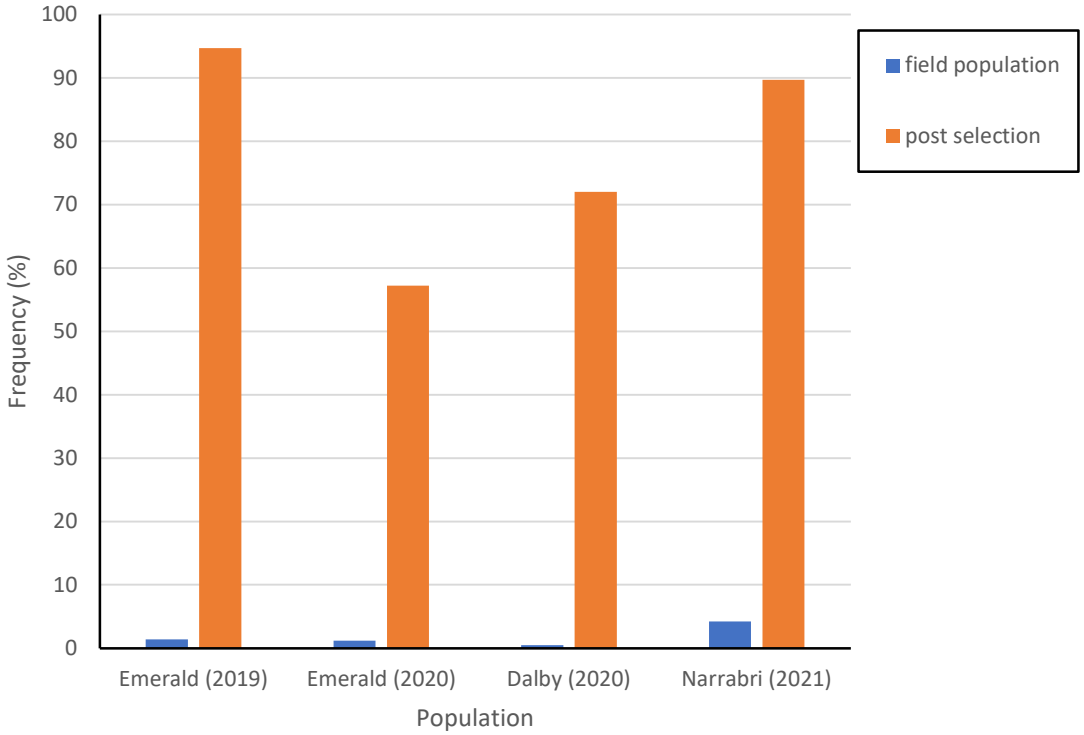

Figure 1. Frequency of the mutation *A2083v* in field populations and post selection (1000–3000 mg/L spirotetramat). Note the frequency of the mutation in the Dalby field population was below the 1% call threshold.

28 – Diamides

Cyantraniliprole

Due to limited use in cotton for silverleaf whitefly control, testing of cyantraniliprole was reduced during 2020 and ceased during 2021. In total 11 populations were tested between 2020 and 2021. The dose response of field populations demonstrate they are highly susceptible to cyantraniliprole with resistance ratios < 1 (Table 18). Resistance was not detected in any of the tested populations.

Table 18. Summary of cyantraniliprole bioassay results

Population	Year	LC_{50} (mg/L)	(95% FL)	Slope (SE)	Mortality (%) at 1 mg/L	Resistance Ratio
Susceptible	-	0.078	(0.065-0.094)	3.31 (0.35)	100	-
Emerald	2020	0.056	(0.045-0.069)	1.46 (0.13)	100	0.72
Theodore	2020	0.035	(0.025-0.049)	1.92 (0.27)	100	0.45
Darling Downs	2020	0.021	(0.017-0.026)	2.92 (0.35)	100	0.27
St George (3)	2020	0.042	(0.029-0.058)	1.54 (0.18)	100	0.54
North Star	2020	0.025	(0.014-0.038)	1.16 (0.18)	100	0.32
Moree (3)	2020	0.017	(0.009-0.028)	1.09 (0.15)	100	0.22
Namoi valley (3)	2020	0.014	(0.006-0.023)	1.34 (0.25)	100	0.18
Narromine	2020	0.043	(0.034-0.055)	2.91 (0.40)	100	0.56
Hillston	2020	0.002	(0.001-0.004)	0.90 (0.15)	100	0.03
Theodore	2021	0.018	(0.007-0.030)	1.15 (0.25)	100	0.23
Emerald	2021	0.019	(0.013-0.027)	1.87 (0.27)	100	0.24

Parasitism assessment

During the 2019/2020 season, three CottonInfo Regional Extension Officers (REOs) participated in this extension activity. The REOs collected leaves from a commercial field and recorded levels of whitefly parasitism for 2 to 4 weeks (Figure 2). A file sharing service was used to share images of whitefly nymphs, which helped with group learning of morphological features used to identify parasitism. The participating REOs gained considerable experience and skills in parasitism

identification. In following seasons, the REOs transferred to using the decision support tool (DST) (Figure 3) but continued to use the file sharing service to share images of nymphs. The participating REOs have used these skills and experience to assist local agronomists in their respective regions, which proved timely given the switch to nymph-based sampling and the use of the DST.

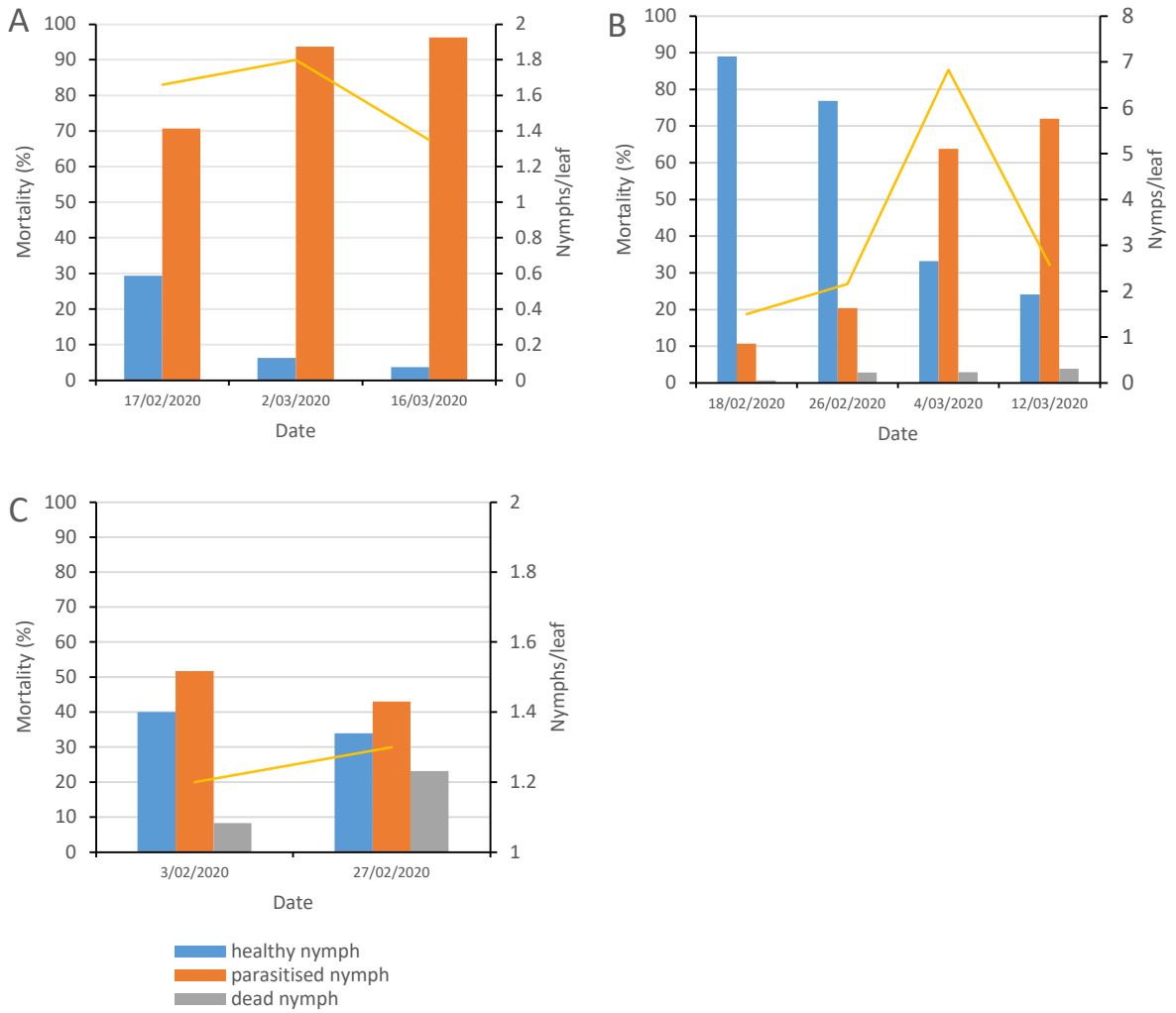

Figure 2. Silverleaf whitefly parasitism assessment by Cotton-Info regional extension officers at Moree (A), St George (B) and Warren (C).

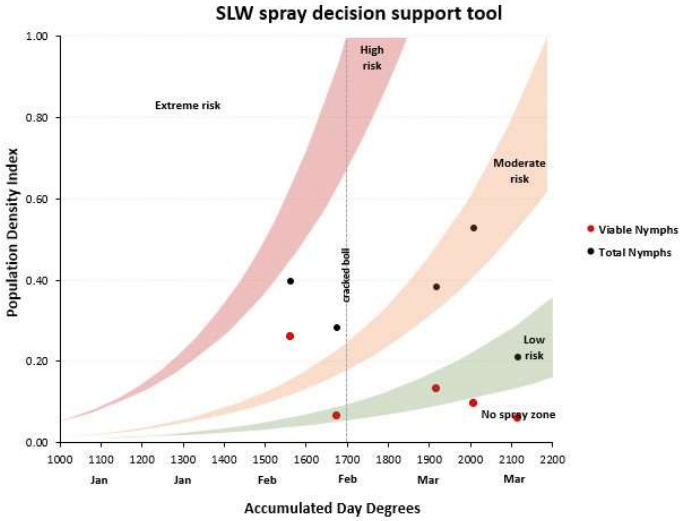

Figure 3. Example of silverleaf whitefly nymph data in the decision support tool, St George 2021.

Review of molecular-based detection of insecticide resistance in silverleaf whitefly

Key Words/Acronyms

AChE:	acetyl choline esterase
cDNA:	complementary DNA
kkv:	protein coding gene in *Drosophila* for chitin synthase enzyme
MEAM1:	*Bemisia tabaci* Middle East-Asia Minor 1 (formerly B biotype)
MED:	*Bemisia tabaci* Mediterranean (formerly Q biotype)
nAChR:	nicotinic acetylcholine receptor
PASA:	polymerase chain reaction allele specific assay
PCR:	polymerase chain reaction
RFLP:	restriction fragment length polymorphisms
RR:	resistance ratio
vgsc:	voltage-gated sodium channel

Background, types of resistance

Resistant insects have mechanisms that are absent or regulated differently from susceptible insects and resistance can be categorised as either non-target site or target-site (R4P Network, 2016).

Non-target site resistance

- behavioural resistance (i.e. avoidance or reduced exposure of the pest to an insecticide)
- reduced penetration of the insecticide via modifications to the physiochemical properties of the pest cuticle, epidermis, or digestive tract
- reduced accumulation of the insecticide at its target site, excretion by transporters (i.e. enhanced efflux)
- intracellular compartmentalisation
- sequestration by molecular binding
- enhanced detoxification
    - enhanced activity (i.e. isoforms more active against the pesticide)
    - overexpression (i.e. overproduction of pesticide neutralising enzymes)
- compensation for the inhibition of the target site by an alternative pathway or enzyme
- neutralisation of cytotoxic molecules generated by the insecticide action

Target-site resistance

- target overexpression: an increase in the concentration of intracellular target protein
- target modification: a structural modification that decreases insecticide binding

Resistance mechanisms are not mutually exclusive; different types of resistance to the same insecticide group can be present in the same insect population, for example populations of *B. tabaci* MED possessing both enhanced detoxification and target site insensitivity, resulting in resistance to pyrethroids (Roditakis et al., 2006).

When resistance involves enhanced detoxification (metabolic resistance), insects use internal enzyme systems to break down insecticides. Three main enzyme families are implicated in detoxification: esterases, glutathione S-transferases (GSTs) and cytochrome P450 monooxygenases (P450s) (Bass and Field, 2011).

Modified target site resistance is where the site that the toxin binds to in the insect has changed via a genetic mutation and is now less sensitive to the toxin. In some cases, target site insensitivity is enhanced by duplication of the genes that encode target-site proteins, reducing the fitness costs associated with homozygous resistance (Bass and Field, 2011).

Bioassays

Detection of insecticide resistance in pest insects has traditionally relied on the use of bioassays that determine the phenotypic response of a population of insects to the toxin. Bioassays can either use a range of doses (concentration gradient) or diagnostic dose(s). Bioassays that use a range of doses can be used to determine the resistance ratio of tested populations. By calculating LC_{50} values and/or minimum effective concentrations (MEC), resistant populations can be separated from susceptible reference populations, thereby confirming the occurrence of resistance and allowing a comparison of the 'strength' of resistance between populations.

Diagnostic or discriminating dose assays can be used to detect resistant populations across a wider geographic range and thereby estimate the frequency of resistant populations within the overall pest distribution.

Biochemical assays can be used to both characterise resistance mechanisms and detect resistance where the mechanism for resistance has been elucidated. Biochemical assays can be used to reveal differences related to the pesticide target (target site resistance) or to pesticide neutralisation (non-target site resistance) (R4P Network, 2016). However, most enzyme or protein assays require the specific pest organs, tissues or subcellular fractions to be analysed, necessitating their careful dissection or preparation prior to testing. For example, a prototype field-based diagnostic lateral flow assay has been developed for whitefly to screen for overexpression of CYP6CM1 (Nauen et al., 2015). This assay has the potential to be used for quick in-field monitoring of resistance.

Molecular screening, and identified mechanisms

Where the mechanism(s) that confer resistance have been discovered, the potential to replace bioassays with molecular screening is an alternative method for screening populations for the presence of resistance.

In the case of metabolic resistance, the molecular method for detecting resistance requires the extraction of RNA from a pools of insects ~100 whitefly adults. First strand cDNA is synthesized by using a primer and reverse transcription kit. The levels of the enzyme (e.g. cytochrome P450) is measured by quantitative PCR (qPCR) amplification (Real-Time PCR Detection System) (Kapantaidaki et al., 2018).

To detect the presence of modified target site resistance, Genomic DNA is extracted. PCR amplification of a diagnostic fragment of DNA using primers is used which can be visualised using gel electrophoresis or sequenced.

1A Organophosphates/ 1B carbamates

Bemisia tabaci resistant to organophosphates have been shown to carry the amino acid change phenylaline (F) to tryptophan (W) in the acetylcholinesterase enzyme ace1 (i.e. the F331W mutation). This mutation of the amino acid results in acetyl choline esterase (AChE) target site insensitivity. The F331W mutation can be detected using PCR-RFLP (Tsagkarakou et al., 2009). Metabolic resistance may also play a role in organophosphate (OP) resistance. The carboxylesterase gene coe1 in *B. tabaci* was found to be overexpressed (approx. 4 fold) in an OP-resistant strain and was demonstrated by quantitative PCR that the elevated expression is not related to gene amplification, but possibly due to modified transcriptional control (Alon et al.,

2008). AChE target site insensitivity and increased esterase activity confers resistance to carbamates as well, i.e. cross resistance between OPs and carbamates (Byrne and Devonshire, 1993).

3A Pyrethroids

In *Bemisia tabaci* the L925I pyrethroid resistance mutation in the para-type voltage gated sodium channel (vgsc) can be used to separate resistant and susceptible populations using PCR-RFLP (Tsagkarakou et al., 2009). Another mutation (T929V) does not create or disrupt a restriction site, however the double nucleotide substitutions (ACT or CTT) can be used to design an allelic specific (PASA) assay (Tsagkarakou et al., 2009). In *B. tabaci* MED resistant strain (GRMAL-RP) from Crete, metabolic resistance has been reported with cytochrome P450-dependent monooxygenase and carboxylesterase activity elevated, while glutathione-S-transferase activity was not different. This strain carried the L925I and T929V mutations of the para sodium channel gene (Roditakis et al., 2006).

4A Neonicotinoids/9B Pymetrozine

The *B. tabaci* cytochrome P450 CYP6CM1 is often found to be over-expressed in populations with imidacloprid resistance (Bass et al., 2015; Karunker et al., 2008). However, there can be populations with high resistance ratios that are not strongly correlated with CYP6CM1 expression level, indicative of additional neonicotinoid resistance mechanisms being present in those populations (Ilias et al., 2015). To detect metabolic resistance RNA sequencing is required. Karunker et al. (2008) used quantitative reverse transcription PCR (RT-qPCR) to investigate the correlation between imidacloprid resistance and the expression level of P450 genes in MED and MEAM1 *B. tabaci.* A TaqMan® gene expression assay (Jones et al., 2011) can be used to routinely monitor for resistance based on the expression levels of CYP6CM1.

Clothianidin and thiacloprid are two other neonicotinoid metabolized by CYP6CM1, while acetamiprid and thiamethoxam are not (Roditakis et al., 2011). Recently it was demonstrated dinotefuran is another neonicotinoid where resistance isn't linked to over-expression of CYP6CM1, as shown by a lack of metabolism of dinotefuran by CYP6CM1 derivatives (Hamada et al., 2019).

Target site resistance to neonicotinoids has been detected in at least two hemipteran species: green peach aphid, *Myzus persicae* and the brown planthopper, *Nilaparvata lugens* (Crossthwaite et al., 2014).

The anti-feedant pymetrozine, which is structurally and functionally unrelated to the neonicotinoid group, shares cross-resistance to imidacloprid via the same detoxification mechanism linked to the over-expression of CYP6CM1 (Nauen et al., 2013).

6 Emamectin benzoate

The efficacy of emamectin benzoate against *B. tabaci* has been studied infrequently (Ahmad and Akhtar, 2018; Ishaaya et al., 2002); as a result, there is no information about potential resistance mechanisms. Elsewhere, resistance mechanisms to emamectin benzoate (EMB) have been best studied in sea lice *Lepeophtheirus salmonis,* which are ectoparasitic copepods of salmon (Poley et al., 2015). Candidate resistance genes were studied, with resistant lice showing significant over-expression of nAChRα7 and down-regulation of nAChRα3 (neuronal acetylcholine receptor subunits). A novel gene candidate LR9 (leukocyte receptor) showed induced expression upon EMB exposure in the resistant males (males are more resistant than females). Other gene candidates like CYP18A1 and peroxinectin did not show similar expression profiles to studies completed on other sea lice populations (Carmichael et al., 2013; Núñez-Acuña and Gallardo-Escárate, 2015). One study that investigated synergism of enzyme inhibitors in *B. tabaci* found an

increase in sensitivity to EMB (5-fold resistant) in field populations when the synergist piperonyl butoxide (inhibitor of AChE activity) was added (Kang et al., 2006). Triphenyl phosphate increased the sensitivity of the field population to a lesser extent, while diethyl maleate did not significantly change the sensitivity of the field population to EMB.

7C Pyriproxyfen

Resistance to pyriproxyfen has been linked to metabolic detoxification, specifically the enzyme activity of P450s and GSTs (Ma et al., 2010). In *B. tabaci* MED after an application of pyriproxyfen to a resistant strain, a cDNA microarray was used to monitor changes in gene expression. In total 111 expressed sequence tags (ESTs) were identified that were differentially upregulated after pyriproxyfen treatment. Of those one, cytochrome P450 CYP9F2 gene was induced 3.85-fold and is likely candidate in insecticide detoxification (Ghanim and Kontsedalov, 2007). In *Trialeurodes vaporariorum* (greenhouse whitefly) the cytochrome P450 gene CYP4G61 has been identified as a strong candidate associated with the resistant phenotype (Karatolos et al., 2012).

9D Afidopyropen

Afidopyropen is similar to pymetrozine (Kandasamy et al., 2017); both belong to group 9, chordotonal organ TRPV channel modulators. As stated above in the section on neonicotinoids, over-expression of CYP6CM1 is known to confer resistance to pymetrozine. At this time, it is not known if over-expression of CYP6CM1 results in detoxification of afidopyropen.

12A Diafenthiuron

Metabolic detoxification was investigated in a lab population of *B. tabaci* MEAM1 that was selected for diafenthiuron resistance in the lab (over 36 generations) with 33-fold resistance (Zhang et al., 2017; Zhang et al., 2016). This research found detoxification genes GST, CYP6CX4, CYP6DW3, CYP6DZ6 and CYP9F were constitutively over-expressed in the resistant (R-DfWf) strain and upregulated following exposure to diafenthiuron. Another detoxification gene CYP6CX1 was not upregulated, and COE1, CYP6CM1 and CYP6A (which were not constitutively overexpressed in the resistant strain) were upregulated following exposure to diafenthiuron. Whether the type of resistance selected in the lab will occur under field conditions remains to be determined. Lab-selected resistance can often be different to the rare mutations that are selected under field exposure to insecticides and should only be used as a guide to potential mutations.

16 Buprofezin

Buprofezin (Group 16) is an inhibitor of chitin biosynthesis in hemipterans and shares the same mode of action as other insecticides including the benzoylureas (BPUs) (Group 15) and etoxazole (Group 10B) (Douris et al., 2016). A mutation (I1042M) in the chitin synthase (CHS1) gene of BPU resistant *Plutella xylostella* was discovered at the same position as the I1017F mutation reported in spider mites that confers etoxazole resistance (Douris et al., 2016). Using a genome-editing CRISPR/Cas9 approach coupled with homology-directed repair in *Drosophila melanogaster* both substitutions (I1056M/F) in the corresponding fly CHS1 gene (kkv) were introduced. Homozygous lines bearing either of these mutations were highly resistant to etoxazole and all BPUs as well as buprofezin.

23 Spirotetramat

Resistance to group 23 insecticides (spirodiclofen, spiromesifen and spirotetramat) has been reported in spider mites, *Tetranychus urticae*, cotton aphid *Aphis gossypii* and whitefly *B. tabaci.* Group 23 insecticides are lipid biosynthesis inhibitors targeting acetyl-CoA carboxylase (ACC)—an enzyme known to catalyze the rate limiting step in fatty acid biosynthesis. Resistance to spirotetramat has been reported in both Spain in *B. tabaci* MED and in Australia in *B. tabaci* MEAM1 (Bielza et al., 2019; Lueke et al., 2020). A recent study has shown that an ACC variant

bearing a mutation (A2083V) results in an amino acid substitution in a highly conserved region of the carboxyltransferase (CT) domain (Lueke et al., 2020). Contribution of the mutation to resistance was confirmed using CRSIPR/Cas9 genome modified *Drosophila* in toxicity assays (Lueke et al., 2020). A pyrosequencing-based diagnostic assay was used to map the spread of resistance alleles in field-collected samples from Spain (Lueke et al., 2020). Recently a Droplet Digital PCR (ddPCR) assay was developed to detect the A2083V mutation in *B. tabaci* (Mavridis et al., 2022).

28 Cyantraniliprole

Cyantraniliprole belongs to the group of insecticide known as diamides, which includes other insecticides like chlorantraniliprole and flubendiamide. These insecticides target insect ryanodine receptors (RyR), which are calcium release channels that are important in calcium homeostasis in numerous cell types. *Bemisia tabaci* (MED) resistance to cyantraniliprole has been reported in China (Wang et al., 2018). A resistant strain CYAN-R (with a RR of 63), was created via repeated backcrossing of the field resistant strain with a susceptible strain. This strain was tested for overexpression of known detoxification related P450 genes, but no significant change in expression levels was detected (Wang et al., 2020). In both studies an adult assay was used in place of the more commonly used nymph-based assay, so it's not possible to directly compare data with our bioassay results. Resistance to diamide insecticides is better understood in diamond back moth (DBM), *Plutella xylostella*. Bioassays of resistant and susceptible DBM demonstrated the resistant population had >1000-fold resistance to both chlorantraniliprole and flubendiamide. Gene sequencing of the proposed diamide binding site of RyR from DBM (resistant from Philippines and Thailand, as well as susceptible strains) were amplified by RT-PCR and sequenced. Comparison of the sequence with susceptible stains revealed non-synonymous mutations in each of the resistant strains that in both cases lead to a glycine to glutamic acid substitution (G4946E) in the protein. A pyrosequencing-based diagnostic assay was developed for resistance monitoring purposes (Troczka et al., 2012). Metabolic mechanisms of resistance have also been proposed, but at this time the research is not conclusive (Troczka et al., 2017).

Confirming mechanisms

As mentioned in the section above covering spirotetramat resistance, gene editing (CRISPR/cas9) can be used to create *Drosophila* strains bearing mutations linked to insecticide resistance. This has so far been used to look at target genes linked to several insecticides including imidacloprid, spinosad, and pyrethroids (Douris et al., 2020). Another method using germ-line transformation (Bischof et al., 2007) has been used to modify *Drosophila* to express pest insect genes, including a carboxylesterase gene (αE7), gluthathione S-transferase gene (GstE2) and a cytochrome P450 gene (Cyp6cm1). Bioassays of these transgenic *Drosophila* confirmed the linkage between these candidate genes to resistance to OPs (diazinon and malathion), DDT and imidacloprid, respectively (Daborn et al., 2012).

Considerations when using molecular based resistance monitoring

The potential advantage of using molecular screening is higher throughput, fast and accurate diagnostics. Hopefully increasing the capability to detect resistance early, prior to significant selection, will allow the deployment of effective resistance management strategies.

The relative strength of and diagnostic value of molecular markers is influenced by the following factors (Van Leeuwen et al., 2020):

- Geographical distribution of the marker (on what scale do resistance mechanisms vary?), i.e. is a marker described elsewhere, is the cause of resistance local or has another mechanism evolved?

- Intensity of underlying resistance phenotype associated with the marker (i.e. how much is the phenotype determined by a single marker?). This has been well studied in *T. urticae* (see (Van Leeuwen et al., 2020) but is less well understood in other pest species.
- Predictive value of the marker for cross-spectrum resistance.
- Epistasis (the effect of a gene mutation is dependent on the presence/absence of mutations in one or more other genes) and how many resistance markers are required for diagnosis in each case.
- Untangling whether gene expression patterns are associated with resistance or host plant (detoxification enzymes can be overexpressed after adaptation to pesticides and plant allelochemicals)
- Understanding of dominance and fitness cost of the resistance marker increases confidence that molecular screening will mirror phenotypic response.
- Robustness, accuracy, and cost effectiveness of the diagnostic assay to capture the marker

In terms of certainty regarding the presence of resistance, molecular-based methods will confirm if the known mechanism is present or not. But they can potentially miss other unknown/novel forms of resistance that haven't been identified (Nauen et al., 2015). The genetic structure of pest whitefly populations can change over the course of a season (Dinsdale et al., 2012) and incursions of resistant genotypes of a pest can potentially carry new resistance mechanisms as seen in *Myzus persicae* (de Little et al., 2017). See the 2015 GRDC final report "Investigation into the possible recent incursion of an insecticide-resistant biotype of green peach aphid into Australia" by Owain Edwards for further information https://grdc.com.au/research/reports/report?id=6823 .

Bioassays detect changes in phenotypic response and can therefore detect resistance without knowledge of the underlying mechanism. But without adequate baseline susceptibility data, interpretation of results, such as separating heritable resistance from vigour tolerance or natural variability can be difficult.

An advantage of full dose response bioassays is the output generated; lethal concentration (LC) values, slope, and resistance factors (difference between a known susceptible population and the tested population at a given LC). Pairing the two approaches, bioassays with molecular techniques is the most reliable way of detecting resistance, particularly in cases where the frequency in the population is still low.

Preservation of insects prior to DNA extraction

Insects preserved for later DNA extraction, are typically stored in 95-99.5% ethanol or acetone, or in a freezer (-20 to -80°C) or combination of these (Nakahama et al., 2019). Alternative preservatives include propylene glycol, RNAlater® and dimethyl sulfoxide (Moreau et al., 2013; Vink et al., 2005).

DNA extraction methods

There are several methods of DNA extraction from insects. The end-use (high-quality sequencing or basic PCR) will guide the method used. The method chosen will also impact the storage life of the extracted DNA.

- **Extraction kits**
  Easy to use extraction kits have been developed to simplify the process. These include the silica-based QIAGEN DNeasy blood and tissue kit in either spin column or 96 well-plates formats.
- **Detergent lysis extraction**
  Lysis extraction is a whitefly DNA extraction procedure based on Barro and Driver (1997).

It uses a Lysis buffer (50 mM KCL, 10 mM Tris-HCl (ph 8.0), 0.45% (v/v) Tween 20, 0.45 (v/v) Triton X, made up to 200 mL with milliQ water and proteinase K). A single preserved whitefly is removed from ethanol and transferred to the side of a 1.5 mL microcentrifuge tube. After the ethanol has evaporated away, 10 µL of lysis buffer is added and the sample homogenised using a pestle (pestles can be made from 500 µl modified pipette tip), before a further 15 µL of lysis buffer is added. The sample is centrifuged then incubated at 65°C for 60 minutes, with a further incubation step at 95°C for 15 minutes (which inactivates the proteinase K). The sample is centrifuged again and then 25 µL of sterile water is added to yield a final homogenate volume of 50 µL.

- **Chelex extraction**
  A cost-effective method of DNA extraction is to use chelex resin (Chelex® 100). Specimens are generally crushed or homogenized (White et al., 2009), however the method can be used for uncrushed specimens (Miura et al., 2017). Specimens are typically placed in 1.5 mL microcentrifuge tubes with 50 µL of chelex and 6 µL of proteinase K then homogenized. The samples are centrifuged and then incubated at 37°C for 1 hour on a dry heating block. To inactivate the proteinase K the samples are incubated at 96°C for 8 minutes. Extracted samples are stored at -20°C; samples should be used within 1-2 months (D. Brookes, personal communication). Prior to use in PCR, defrost, vortex and centrifuge at full speed for 20 minutes. Always pipette from the very top of the solution to ensure chelex is not taken up into the PCR, as it inhibits the reaction and other downstream applications.

PCR

PCR makes it possible to specifically address a particular DNA sequence and amplify it to extremely high copy numbers, making it possible to study in detail. PCR combines the unique attributes of being very sensitive and specific with a great degree of flexibility. The PCR reaction uses a few standardised components:

- Water
- PCR Buffer
- MgCL2
- dNTPs
- Forward Primer
- Reverse Primer
- Target DNA
- Polymerase (TAQ)

Species ID

To separate between the two endemic Australian *B. tabaci* (AUS I and AUS II), Wongnikong et al. (2019) used Chelex extraction (as described above). PCR was used to amplify a 819 bp region of the mtCOI gene, using the primer C1-J-2195 (5'–TTGATTTTTTGGTCATCCAGAAGT-3') and L2-N-3014 (5'-TCCAATGCACACTTAATCTGCCATATTA-3') (Simon et al., 1994).

Each 30 µl reaction contained 2 µl DNA template, 1U MyTaq Polymerase (Bioline, Australia), 0.2 µM of each PCR primer and 1x buffer. PCR reaction conditions consisted of an initial denaturation at 95°C for 3 min, followed by 10 cycles of 30 s at 95°C, annealing at 45°C for 30 s, and 1 min extension at 72°C, then 30 cycles of 30 s at 95°C, annealing at 50°C for 30 s, and 1 min extension at 72°C, and the final extension was at 72°C for 10 min. PCR products were verified by agarose gel electrophoresis and cleaned using 1 U of Exonuclease I and Antarctic Phosphatase (New England Biolabs, Ipswich, Mass., USA) by incubating at 37°C for 20 min followed by 10 min enzyme

denaturation at 80°C. The clean products were sequenced using the same forward and reverse primers used for PCR, by Macrogen Inc. (Seoul, Republic of Korea).

Sequences were aligned with representative B. tabaci mtCOI haplotypes (from the National Center for Biotechnology Information (NCBI)) plus some unpublished sequences of Australian B. tabaci (S.L. van Brunschot, unpublished), using MUSCLE, and checked for premature stop codons, indels and frameshift mutations (indicators of pseudogenes) by manually checking all nucleotide sequences, including the translation of each sequence, in Geneious version 9.1.8 (http://www.geneious.com). The alignment was trimmed to 655 bp and a Bayesian tree was constructed with MrBayes (using the GTR + I + G model after checking for the best model with jmodeltest (Darriba et al., 2012; Guindon and Gascuel, 2003). in Geneious version 9.1.8using 100,000 iterations for the burn-in followed by 1,000,000 iterations.

Detecting mutations SNPS

PCR can also be used to detect genomic mutations that result in resistance to insecticides. For example the diagnostic assay developed by Tsagkarakou et al. (2009) for detecting pyrethroid resistant *B. tabaci*. It distinguishes between resistant (I925 or V9292) and susceptible (L925 or T929) para-type voltage gated sodium channel alleles. The diagnostic assay for the detection of the L925I mutation is based on the PCR amplification of a 184 bp fragment within the IIS4-5 linker of the *B. tabaci* sodium channel gene, using the allele specific primers Bt-kdrF1 (5'-GCCAAATCCTGGCCAACT-3') and Bt-kdr-RIntr1 (5'-GAGACAAAGTCCTGTAGC-3'), and subsequent restriction digestion with the enzyme DdeI. DdeI recognises one site in the amplified fragment of the susceptible allele (L925), and the digestion yields two fragments of 124 and 60 bp.

RNA

Quantitative reverse transcription PCR (RT-qPCR) is used when the starting material is RNA. In this method, RNA is first transcribed into complementary DNA (cDNA) by reverse transcriptase from total RNA or messenger RNA (mRNA). The cDNA is then used as the template for the qPCR reaction.

Future directions

There are several new developments in molecular diagnostics that may change how resistance monitoring is conducted.

Droplet digital PCR (ddPCR) is the a recent development in PCR that can be used to accurately detect target site mutations with a larger pooled sample of non-target DNA (Mavridis et al., 2022). Droplet digital PCR is a digital PCR method utilising a water-oil emulsion droplet system. Droplets are formed in a water-oil emulsion to from the partitions that separate the template DNA molecules. The droplets serve essentially the same function as individual test tubes or wells in a plate in which the PCR reaction takes place – but in much smaller format. The massive sample partitioning is a key aspect of the ddPCR technique.

The Droplet Digital PCR System partitions nucleic acid samples into thousands of nanoliter-sized droplets, and PCR amplification is carried out within each droplet (Hindson et al., 2011). This technique has a smaller sample requirement than other commercially available digital PCR systems, reducing cost and preserving precious samples.

Benefits of ddPCR (as reported by the manufacturer bio-rad https://www.bio-rad.com/en-au/life-science/learning-center/introduction-to-digital-pcr/what-is-droplet-digital-pcr?ID=MDV31M4VY#Hindson). ddPCR technology enables high-throughput digital PCR in a manner that uses lower sample and reagent volumes and reduces overall cost compared with

other methods while maintaining the sensitivity and precision that are the hallmarks of digital PCR. The benefits of ddPCR technology include:

- Absolute quantification — ddPCR technology provides an absolute count of target DNA copies per input sample without the need for running standard curves, making this technique ideal for measurements of target DNA, viral load analysis, and microbial quantification
- Unparalleled precision — the massive sample partitioning afforded by ddPCR enables the reliable measurement of small fold differences in target DNA sequence copy numbers among samples
- Increased signal-to-noise ratio — high-copy templates and background are diluted, effectively enriching template concentration in target-positive partitions, allowing for the sensitive detection of rare targets and enabling a ±10% precision in quantification
- Removal of PCR bias — error rates are reduced by removing the amplification efficiency reliance of qPCR, enabling the detection of small (1.2-fold) differences
- Simplified quantification — neither calibration standards nor a reference (the $\Delta\Delta$Cq method) is required for absolute quantification
- Reduced consumable costs — reaction volumes are in the pico- to nanoliter ranges, reducing reagent use and the sample quantity required for each data point
- Lower equipment costs — the emulsion-based reaction system means that the PCR reactions can be performed in a standard thermal cycler without complex chips or microfluidics
- Superior partitioning — ddPCR technology yields 20,000 droplets per 20 µl sample, nearly two million partitioned PCR reactions in a 96-well plate, whereas chip-based digital PCR systems produce only hundreds or thousands of partitions. The greater number of partitions yields higher accuracy

Loop-mediated isothermal amplification (LAMP) is an alternative method to PCR that amplifies DNA with high specificity, efficiency, and rapidity under isothermal conditions (Notomi et al., 2000). The advantages of LAMP are it has rapid and simple protocols, minimum equipment required, can be deployed under field conditions, while its shortcomings include difficulty in multiplexing, it's only a qualitative method and it has lower specificity and sensitivity in allele detection compared to other more advanced methodologies (e.g., pyrosequencing) (Vontas and Mavridis, 2019). Allele-specific LAMP (AS-LAMP) has been used to detect pyrethroid resistance in *Anopheles gambiae* and could discriminate between wild-type homozygote, heterozygote and resistant homozygote within 75 minutes (Badolo et al., 2012). The AS-LAMP method has the advantage of being faster and at least as sensitive and specific as the AS-PCR method (Badolo et al., 2012). LAMP has also been used to identify *B. tabaci* (Blaser et al., 2018) and distinguish between members of the *B. tabaci* cryptic species complex, e.g. MEAM1 and MED (Hsieh et al., 2012).

Other 'field-friendly' technologies include the Oxford Nanopore MinION (Boykin et al., 2019) and portable qPCR platforms e.g. Genedrive® (Unwin et al., 2018) have been demonstrated to be capable of point-of-need field diagnostics, but both require significant capital investment.

High-throughput multiplex real-time PCR assays (Taqman, High Resolution Melt (HRM) and Melt-Curve Assay (MCA)) are fluorescence-based 'closed-tube' assays that can be used to genotype pooled individuals without post-PCR processing (gel-electrophoresis) (Vezenegho et al., 2009)

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Discussion

Surveillance of potential exotic *Bemisia* found no evidence of *B. tabaci* MED in populations collected from cotton production regions between 2013 and 2021. Evidence of *B. tabaci* ASIA II in Australia has been recently published, with detections reported in Darwin, Kununurra, and Emerald (Wongnikong et al., 2021). *B. tabaci* ASIA II was not detected in our samples, but Emerald was the only region where surveillance overlapped with Wongnikong et al., (2021). As the cotton industry establishes into new regions, particularly in Northern Australia, increased surveillance in these regions will be required.

Using both bioassay and DNA sequencing, the level of resistance to pyrethroids in silverleaf whitefly populations collected in 2020 and 2021 was determined. Almost all populations had resistance with frequencies between 1.1 and 4.7% The two techniques were not 100% correlated but given the low frequency of resistance, the results are reliable and suggest that the discriminating dose of 300 mg/L is accurate. These results form part of a larger, recently published study (Fang et al., 2022) that was completed in collaboration with CSIRO and the University of Canberra.

In 2020, the project looked at the toxicity of imidacloprid to silverleaf whitefly collected from cotton. The initial aim was to develop baseline susceptibility data, instead, we found widespread resistance to imidacloprid in many of the tested populations. Testing of the lab resistant strain that was established in 2015 from Griffith (selected with clothianidin), revealed strong cross-resistance between imidacloprid, thiacloprid, thiamethoxam and clothianidin when tested with a foliar assay. Cross-resistance was less pronounced when a systemic assay was used. In the resistant strain, low level cross-resistance was observed to acetamiprid. Interestingly testing to date of field populations has not detected clear evidence of resistance to acetamiprid. Based on testing of our neonicotinoid-resistant lab strain and published literature there is no cross-resistance between imidacloprid and dinotefuran. No evidence of resistance to dinotefuran was observed in any of the populations tested in the past three years. Cross-resistance between imidacloprid and pymetrozine was observed in 2020 and is well documented in the scientific literature. It can be surmised that the use of neonicotinoids like imidacloprid or clothianidin could favour SLW population increases by disrupting natural enemies if used to control other pests (e.g., clothianidin to manage *Nezara viridula*).

Whitefly resistance to pyriproxyfen remains a concern, with populations being detected with resistance despite the introduction of an application window designed to reduce selection pressure. Initial results were promising with a significant decline in the number of populations detected with resistance, particularly during 2018/19 and 2019/20. However, in more recent seasons (2020/21 & 2021/22) there has been an increase in the number of populations where resistance was detected, and it does not appear to be linked to usage in cotton. Data collected in 2021 (CCA annual market audit) showed industry-wide use of pyriproxyfen was low, and use has been steadily declining since the 2017/18 season. Year to year variability in the detection of resistance in populations is therefore hard to explain. Reducing selection pressure through the introduction of the application window does rely on the assumption that fitness costs are associated with insecticide resistance (Freeman et al., 2021). If this is not correct for pyriproxyfen resistant *B. tabaci* as speculated by Crowder et al. (2009) than the resistance allele frequency may stabilise at a level where resistance is retained, particularly if there is an absence of susceptible migrants. This may explain the recent results but would require further quite challenging research to resolve.

Low level resistance to spirotetramat was detected from several regions in the past three years. Bioassay results are supported by DNA sequencing that showed the frequency of the mutation was between 1 and 4%. This finding has been extended to industry and proactive changes were made to the IRMS with the aim of reducing selection pressure. Based on analysis of CCA market audit data, spirotetramat usage has seen a rapid increase. Extension of these research findings, which highlight the potential risk of resistance developing to spirotetramat must continue.

Buprofezin was registered in 2020 and has the potential to be used in place of either spirotetramat or pyriproxyfen. Like pyriproxyfen it is an insect growth regulator, but is specific to homopteran pests (whitefly, leafhoppers, scale insects), so has low impact on most natural enemies. Compared to pyriproxyfen and spirotetramat it has limited translaminar activity and instead relies on contact and vapour activity. Results collected over the past three years show no sign of pre-existing resistance to buprofezin. Resistance has been detected in other countries where buprofezin has been used to control whitefly, so overreliance needs to be avoided and reflecting this sentiment, a restriction on it use was added to the IRMS shortly after its registration.

Testing of silverleaf whitefly with diafenthiuron, emamectin benzoate, and cyantraniliprole over the past three years show no changes, with no evidence of resistance detected. Baseline susceptibility of silverleaf whitefly to afidopyropen and flupyradifurone was evaluated and this data can be called upon if changes are made to their registration.

A review of molecular-based methods for resistance screening was completed and has proven valuable for the adoption of these approaches in recent research. To date ethanol samples of > 80 field populations have been sequenced to determine the frequency of mutations in *ace1*, *vgsc,* and *ACC* genes that are linked to OP/carbamate, pyrethroid and spirotetramat resistance, respectively.

Parasitism of silverleaf whitefly by wasps including *Eretmocerus hayati* can be substantial and provide meaningful biological control. However, parasitism in whitefly can be difficult to detect and the skill of categorising nymphs as healthy or parasitised is valuable, particularly now sampling is based on temporal changes in nymph population density. As an extension exercise, CottonInfo regional extension officers (REOs) participated in training where they evaluated parasitism in a field in their region. Project staff provided support in learning the identification skill. Having gained experience in identifying parasitism, these REOs have been able to aid local agronomists as they adopt the new nymph-based decision support tool.

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Part 4 – Summary for public release

This summary is designed to provide a short overview of the project for all interested parties. It will be published on Inside Cotton, CRDC's digital repository, along with the full final report (if suitable for public release). The summary may also be published on grow[AG], a collaborative platform that showcases Australian agrifood research, development, and extension projects that are current or have been completed since 1 July 2018. Please complete all fields, ensuring that this exceeds no more than two pages.

Project title:	**Sustainable SLW management through improved insect resistance monitoring**	
Project details:	CRDC project ID:	DAQ2001
	CRDC goal:	1. Increase productivity and profitability on cotton farms
	CRDC key focus area:	1.3 Protection from biotic threats and environmental stresses
	Principal researcher:	Dr Jamie Hopkinson, Senior Research Scientist
	Organisation:	Department of Agriculture and Fisheries, Queensland Government
	Start date:	1/07/2019
	End date:	30/06/2022
Objectives	• Monitoring of insecticide resistance in silverleaf whitefly • Biosecurity surveillance of whitefly in cotton production regions • Silverleaf whitefly parasitism extension	
Background	Silverleaf whitefly are a major pest of cotton that must be managed to limit their contamination of cotton with honeydew (sticky cotton). As this pest can readily evolve resistance from insecticides exposure, they must be managed in a way that minimises this risk. Data collected from this project is used to make informed changes to the insecticide resistance management strategy (IRMS) for the cotton industry with the aim of providing sustainable management options for silverleaf whitefly	
Research activities	Annual insecticide resistance testing was conducted on silverleaf whitefly collected from cotton production regions. Insecticides included pyriproxyfen, spirotetramat, buprofezin, bifenthrin and acetamiprid. Species composition of collected whitefly was determined using DNA barcoding. Populations were sequenced for the presence of known resistance mutations to pyrethroids, organophosphates and spirotetramat. An extension training exercise on whitefly parasitism was completed with CottonInfo regional extension officers.	
Outputs	The project annually reported the insecticide resistance status of silverleaf whitefly and where applicable suggested appropriate changes to the IRMS to CRDC, Cotton Australia via the TIMS IRMS technical panel. Other industry outputs included presentations at industry extension events (CSD cotton management tour), extension articles (Spotlight, Australian cottongrower, and extension factsheets for the CottonInfo extension network. Research was presented at the Australian Cotton Research Conference, October 2019 and in three scientific journal publications.	

Impacts	Annual monitoring of insecticide resistance in whitefly has forewarned the industry of the emergence of resistance to spirotetramat, and restrictions in use have been implemented via the IRMS which aim to slow the evolution of resistance to this highly effective and IPM-compatible insecticide. This project has provided data to support the ongoing use of a '30-day spray window' for pyriproxyfen that has helped reduce the selection pressure that pyriproxyfen was being exposed to. While data from this project shows resistance to pyriproxyfen is an ongoing issue, resistance levels as measured by LC_{50} have stabilised. Awareness of the value of parasitoids as natural sources of whitefly population suppression was extended to the industry via training of CottonInfo Regional Extension Officers, providing them with the skill to assist agronomists or growers in their region identify the activity of parasitoids in fields they are managing.
Key publications	<u>CottonInfo factsheets</u> Resistance monitoring 2020/21 season Managing silverleaf whitefly in Australian cotton <u>Conference presentation</u> Hopkinson, J., Pumpa, S., van Brunschot, S., Fang, C., Frese, M., Tay, W. T., and Walsh, T. (2019) Insecticide resistance status of *Bemisia tabaci* MEAM1. *Australian cotton research conference*, University of New England, Armidale. <u>Scientific articles</u> Fang, C., Hopkinson, J. E., Balzer, J., Frese, M., Tay, W. T., and Walsh, T. (2022) Screening for insecticide resistance in Australian field populations of Bemisia tabaci (Hemiptera: Aleyrodidae) using bioassays and DNA sequencing. *Pest Management Science* 78, 3248-3259. Hopkinson, J., Pumpa, S., van Brunschot, S., Fang, C., Frese, M., Tay, W. T., and Walsh, T. (2020) Insecticide resistance status of Bemisia tabaci MEAM1 (Hemiptera: Aleyrodidae) in Australian cotton production valleys. *Austral Entomology* 59, 202-214. Lueke, B., Douris, V., Hopkinson, J. E., Maiwald, F., Hertlein, G., Papapostolou, K. M., Bielza, P., Tsagkarakou, A., Van Leeuwen, T., Bass, C., Vontas, J., and Nauen, R. (2020) Identification and functional characterization of a novel acetyl-CoA carboxylase mutation associated with ketoenol resistance in Bemisia tabaci. *Pesticide Biochemistry and Physiology* 166. 104583