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AgriFutures® Pasture Seeds

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Foreword

Pasture seeds are a crucial industry that underpins productive and profitable animal production (i.e. meat, milk and wool production). Australian pasture seed is predominately grown in South Australia, but key growing areas span Tasmania, New South Wales, Victoria and the southern part of Western Australia. Australian pasture seed is exported across the world, with Europe and the USA key importers of lucerne and clover seed. Certified lucerne production makes up over 60% of the total levied temperate pasture seed produced.

Pest management will always remain a key priority for pasture seed growers, but with the shifting global headwinds and evolving markets, there is an even bigger priority to ensure the tools the growers have continue to serve them. Especially as pest occurrence and impact will only increase as the climate changes.

Previous work from Cesar identified that populations of insecticide-resistant bluegreen aphid (BGA) were appearing in South Australia and posed a significant threat to the pasture seed industry. BGA represent significant crop loss as they directly feed on the plant and spread harmful plant viruses. This project, done in conjunction with GRDC's own investment in BGA insecticide resistance and management for the grain industry sought to:

- Gather information on the spread and variation of insecticide resistance in the field
- Generate baseline data on the biocontrol option for BGA
- Develop pest-management guidelines to support extension efforts by Lucerne Australia.

This project identified that regular monitoring for BGA remains crucial for its control as accurate identification of the species dictates the most effective chemical control. Effective chemical control and optimal application, in combination with rotating modes of action, also benefits natural predators of the BGA and reduces the risk of resistance.

BGA has yet to develop resistance to the newer insecticides like sulfoxaflor and flonicamid, meaning these should be used in regions with highly resistant populations (where organophosphates and pyrethroids should be avoided).

Agronomists and growers alike should observe the areas of highly resistant populations and incorporate the findings and recommendations from this report in the application of integrated pest management to manage the increasing occurrence of insecticide resistance.

This project was completed as part of the AgriFutures Pasture Seed Program, which aims to support a thriving and collaborative Australian certified temperate pasture seeds industry. For more information and resources, visit agrifutures.com.au/rural-industries/pasture-seeds/

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About the authors

Dr Evatt Chirgwin is a Senior Research Scientist at Cesar Australia. His research focuses on the challenges that insecticide-resistant pests, including insects and mites, pose to Australian crops and how growers can employ novel IPM-based strategies to tackle these pests. Evatt previously led the initial discovery of insecticide resistance in the bluegreen aphid. He is also involved in research on novel biocontrol methods using parasitoids and predators and exploring novel approaches to slow the insect-mediated spread of plant viruses. Evatt managed this project, designed and conducted bioassays and undertook all the data analysis.

Lilia is an extension scientist at Cesar Australia. She has a background in agricultural science and experience developing extension strategies, resources and stakeholder engagement activities across a range of pest-management projects including extension for this project.

Karyn Moore is a Research Scientist at Cesar Australia. She manages the entomology and pesticide laboratories and maintains the aphid cultures. She conducted or assisted with all bioassays in this project.

Dr Aston Arthur is a Research Scientist at Cesar Australia who is passionate about pest management and has a long history of developing effective and sustainable control options for agricultural pests. She has worked on a range of agricultural pests, in particular mite pests, for almost 20 years. Aston has considerable experience in designing and running chemical bioassays and microcosm trials on a range of pests including aphids, mite pests and lucerne flea. She was involved with some of the insecticide bioassays undertaken on this project.

Danielle Lannin England is the Executive Officer for Lucerne Australia. Danielle has worked in agricultural extension for more than 25 years and brings to the lucerne seed industry a passion for feedbase/pasture production RDE.

Associate Professor Paul Umina is a director of Cesar Australia and a Melbourne Enterprise Fellow at The University of Melbourne. Paul has been an active researcher in agricultural pest management and insecticide resistance for more than 20 years.

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Abbreviations

Abbreviation	Description
BGA	Bluegreen aphid
CI	Confidence interval
DF	Degrees of freedom
IAG	Industry advisory panel
IPM	Integrated pest management
IRMS	Insecticide resistance management strategy
LC	Lethal concentration
MoA	Mode of Action
NSW	New South Wales
SA	South Australia
VIC	Victoria
WA	Western Australia

Contents

Executive summary

Bluegreen aphids (BGA; *Acyrthosiphon kondoi*) are a global pest of pastures and legume crops. Historically, Australian growers have relied on insecticides as an effective and economical method for managing BGA. However, in 2021, three populations of BGA from South Australia (SA) and New South Wales (NSW) were found to have evolved resistance to organophosphates, carbamates and synthetic pyrethroid insecticides. This was the first documented case of insecticide resistance in BGA worldwide, raising concerns for the management of this pest within the pasture and pasture seeds industry. To address these concerns, project PRO-015983 was initiated with three main objectives. First, a resistance surveillance program monitored the geographic distribution of insecticide-resistant BGA populations across southern Australia and the magnitude of resistance exhibited by these populations. Twenty-one new insecticide-resistant populations were identified, and resistant BGA were discovered in several new regions including the Eyre Peninsula region in SA, Tamworth in NSW and several locations across Victoria. The majority of the resistant BGA populations were present in lucerne pastures and seed crops within the main lucerne seed production region in south-eastern SA where nearly every population tested showed resistance. This project also identified insecticide-resistant BGA on some crop types for the first time including lentils, sub-clover and vetch. BGA populations displayed different degrees of resistance to the three insecticides tested here, typically showing less resistance to carbamates compared to organophosphates and pyrethroids. Consequently, carbamate insecticides may still provide control of BGA if applied under optimal conditions (most importantly, temperature). Our second objective focused on generating baseline data on natural enemies that provide biocontrol of BGA. To do this, we surveyed parasitoid wasps attacking BGA across southern Australia and conducted sticky trap surveys on potential generalist predators of BGA present in lucerne seed paddocks. We found that *Aphidius ervi* was the only species attacking BGA, irrespective of region or crop type. This surprising lack of parasitoid diversity presents both opportunities and challenges for future biocontrol of BGA. We subsequently found through laboratory-based studies, *A. ervi* can also aid in the biocontrol of other aphids in pasture seed crops including cowpea aphids (*Aphis craccivora*), pea aphids (*Acyrthosiphon pisum*) and spotted alfalfa aphids (*Therioaphis trifolli*). Our sticky trap survey identified several generalist aphid predators (including ladybirds, lacewings, rove beetles and hoverflies) that may also help provide control of BGA. Our third objective was to develop management recommendations and communicate our research findings to the pasture and pasture seeds industry through field day presentations, webinars, scientific articles, workshops, podcasts and radio. The project team developed management recommendations and future research priorities for BGA with guidance from an industry advisory panel of expert agronomists from the pasture seed industry. These management recommendations and future research priorities covering monitoring methods, biocontrol, insecticides and cultural control are outlined at the end of this report.

Introduction

The Australian pasture seed industry provides a fundamental resource for pasture-based agriculture both locally and abroad (Carter and Heywood 2008, Hudson 2017, Oliver et al. 2018). The industry includes around 500 growers located in southeastern Australia and southwestern WA (Oliver et al. 2018). Lucerne is the most predominant seed production crop, accounting for approximately 60% of certified pasture seed produced (Oliver et al. 2018). As with many agricultural industries, pasture seed growers contend with persistent challenges posed by invertebrate pests (insects and mites) that damage crops and reduce yields (Allen 1989, Ryalls et al. 2013). These pests present a dynamic challenge for pasture seed growers as growers must manage a variety of pests whose risks fluctuate from year to year due to local factors (e.g. climate) (Hoffmann et al. 2008, Maino et al. 2018, Umina et al. 2021a). Therefore, effective and sustainable pest-management strategies are crucial for protecting the productivity of the pasture seed industry.

Bluegreen aphids (BGA; *Acyrthosiphon kondoi*) are major pests of lucerne, medic, clover, pulses and mixed pastures in Australia and several other countries (Bailey 2007, Humphries et al. 2012, Clouston et al. 2016). BGA reduces crop growth and yield through feeding (primarily on upper leaves, stems and terminal buds) and secreting bioactive compounds into plants (Edwards et al. 2008, Chirgwin et al. 2024). BGA also spreads multiple plant viruses within and between crop types including cucumber and alfalfa mosaic virus (Ryalls et al. 2013, van Leur et al. 2021). Outbreaks of BGA in crops can escalate rapidly due to their quick generation time (~10 days) and ability to disperse rapidly via winged morphs. Thus, growers must proactively manage this pest to minimise yield losses (Lawrence 2009).

Australian pasture seed growers have primarily relied on insecticides to manage BGA (Humphries et al. 2016, Chirgwin et al. 2024), a strategy that has historically offered reliable and cost-effective control. However, a sustained reliance on a limited group of insecticides has applied strong selection pressure on BGA populations, resulting in the emergence of insecticide resistance in this species. In 2021–22, research identified three BGA populations in lucerne paddocks in South Australia (SA) and New South Wales (NSW) that had developed resistance to all three insecticide Modes of Action (MoA) — organophosphates (Group 1A), carbamates (Group 1B) and synthetic pyrethroids (Group 3A) — used for their control (Umina et al. 2019, Chirgwin et al. 2022). Concerningly, this marked the first documented cases of insecticide resistance evolving in this species globally, raising concerns about the potential risks this new challenge poses to the pasture seed industry and the uncertainty surrounding effective management strategies for BGA.

To address the emerging concerns regarding insecticide-resistant BGA and to mitigate the risk of further resistance development in BGA, project PRO-015983 was established with three primary objectives: 1) To monitor the geographic distribution and magnitude of resistant BGA across southern Australia through a resistance surveillance program; 2) Generate baseline data on natural enemies providing biocontrol of BGA and 3) Develop management recommendations and communicate research findings to stakeholders.

Objectives

1) Insecticide resistance surveillance program

We established a surveillance program to measure temporal shifts in the distribution and magnitude of insecticide resistance in BGA populations across southern Australia. BGA populations were collected and screened for resistance using laboratory bioassays. This objective helped identify the regions where insecticide-resistant BGA pose a risk, allowing more accurate and effective management recommendations for growers. In addition to the surveillance program, baseline sensitivity data were generated for three alternative insecticide modes of action that may be used for future BGA control. The baseline data will also provide a valuable reference for future insecticide resistance monitoring to these chemicals.

2) Improve baseline biocontrol options for BGA

Biocontrol can assist in managing invertebrate pests while reducing reliance on insecticides and the associated selection pressure that drives insecticide resistance to evolve. However, there is currently a lack of knowledge on the natural enemies that attack BGA in pasture seed crops. In this project, we gathered baseline data on a key group of natural enemies of aphids: parasitoid wasps, which deposit larvae inside (and subsequently kill) aphids. This was undertaken by assessing the BGA populations collected through the surveillance program to determine which parasitoid species were attacking BGA across the different regions and crops. We also worked with local agronomists to survey generalist aphid predators found within lucerne seed crops.

3) Communication and extension

We communicated research findings and pest management recommendations to the industry throughout the life of the project using multiple platforms (e.g. presentations, articles, social media, videos, radio, and podcasts). Based on the project findings, we developed management guidelines alongside an advisory group of expert agronomists from the pasture seed industry to help growers manage BGA populations and mitigate the risk of resistance evolving further in the future.

Parallel Grains project

PRO-015983 was run alongside a parallel project, CES2208-001RTX, funded by the Grains Research and Development Corporation (GRDC). For resistance management strategies to be most effective against any pest, they must consider the selection pressures present across all industries that manage that pest. Given that BGA affects both pasture and grain crops (mainly pulses), this cross-industry collaboration enabled the development of more integrated and effective management strategies tailored to the needs of both industries. Through these parallel projects, we assessed whether insecticide-resistant BGA populations in pulse crops pose challenges to pasture seeds and vice versa. Additionally, this partnership enabled valuable cross-industry exchange, leading to more coordinated and better outcomes for managing BGA. In turn, this cross-industry collaboration will enhance how growers across both industries manage BGA by not only improving management of current BGA populations but also reducing the risk of resistance developing further or spreading to new insecticides in the years to come.

Methodology

Objective 1: Insecticide resistance surveillance program

The resistance surveillance program mapped the distribution of insecticide-resistant BGA across southern Australia. BGA populations were tested for resistance to the three chemical Mode of Action (MoA) groups this species had previously evolved resistance: organophosphates (Group 1B), carbamates (Group 1A), and synthetic pyrethroids (Group 3A). We also generated baseline sensitivity data for three other insecticide MoA: flupyradifurone (Group 4D), sulfoxaflor (Group 4C) and flonicamid (Group 29), which may be considered for future BGA management.

Aphid populations and culturing

40 BGA field populations were collected and tested as part of the resistance surveillance program (Fig.1; Table 1). Twenty-six populations were collected from pasture and pasture seed crops, and 14 populations from pulse crops (Fig.1). Collections were prioritised in areas where chemical control failures were reported (e.g. South Australia–Victorian border). We included a known insecticide-susceptible BGA population, originally collected in 1999 from Western Australia (WA) and maintained in the lab as a standard for all bioassays. All BGA field populations were collected by the project team or sent in by growers and agronomists.

Figure 1. Map of collection sites for all the BGA field populations tested in the resistance surveillance program. BGA collected from pasture and pasture seed sites are shown in purple, while those collected from grain crops are shown in green.

Upon arriving in the laboratory, aphids from each population were separated into 55mm petri dishes filled with 1% (w/v) agar, each containing three lucerne leaves and positioned inside a sealed mesh container. The aphids were subsequently kept in a controlled temperature room at 20°C for 14 days to remove any parasitoid wasps or pathogenic fungi (Umina et al. 2014). An isofemale line was then established and cultured from each population in petri dishes containing 1% (w/v) agar with lucerne leaves. The petri dishes were maintained in a controlled temperature (CT) cabinet at 11°C with a L14:D10h photoperiod. Aphids were transferred to dishes with fresh leaves every ~10 days.

Prior to testing, each BGA population was set up into bulking colonies to obtain sufficient aphids for the bioassays. To create bulking colonies, aphids were moved from laboratory cultures to lucerne plants grown in pots (50x120x50mm) filled with potting mix and placed inside a separate exclusion cage. These exclusion cages were held within a CT room maintained at 20°C with a L16:D8h photoperiod. The bulking colonies reproduced for four weeks, resulting in >600 adult aphids per population.

Prior to each bioassay, we ensured all aphids tested were of similar age and development stage. To do so, we transferred adult aphids from the bulking colonies to petri dishes containing lucerne leaves in

1% (w/v) agar and placed them in a CT room at 20°C to produce offspring. The aphids reproduced, and 3–4-day-old offspring were used in the bioassays.

Table 1. Details of all BGA populations collected and tested during the current project.

Location	State and region	Date collected	Crop	Latitude	Longitude
Albany	WA – south coastal	10/23	Clover	-34.48	118.48
Avalon	Vic – southern	5/24	Lucerne	-38.02	144.49
Bangham	SA – upper southeast	9/21	Sub-clover	-36.49	140.88
Banyena	Vic – Wimmera	10/22	Lentils	-36.55	142.82
Brimbago	SA – upper southeast	8/22	Lucerne seed	-36.23	140.32
Canowindra	NSW – Central Tablelands	10/20	Lucerne	-33.58	148.63
Coolah	NSW – Central West	9/24	Lucerne	-31.82	149.72
Coombe	SA – upper southeast	8/22	Lucerne seed	-35.95	140.32
Coulta	SA – Eyre Peninsula	9/23	Lucerne	-34.46	135.46
Crowlands	Vic – central	9/23	Lucerne	-37.08	143.05
Culburra	SA – upper southeast	10/22	Lucerne seed	-35.82	140.11
Cummins	SA – Eyre Peninsula	10/22	Lucerne	-34.46	135.46
Dhuragoon	NSW – Riverina	9/23	Medick	-35.10	144.03
Euberta	NSW – Riverina	5/23	Lucerne	-35.09	147.26
Eudunda	SA – northeast	9/22	Vetch	-34.08	139.00
Hinks	SA – Eyre peninsula	9/23	Vetch	-33.99	135.86
Jung	VIC – Wimmera	10/22	Lentils	-36.55	142.36
Kaniva	VIC – Wimmera	9/23	Lentils	-36.23	141.14
Kapinnie	SA – Eyre Peninsula	9/23	Lucerne	-34.16	135.50
Keith†	SA – upper southeast	12/20	Lucerne seed	-36.10	140.37
Kerang	Vic – Mallee	9/23	Lentils	-35.96	143.66
Laanecoorie	Vic – central	6/23	Lucerne	-36.79	143.96
Lillimur	Vic – Wimmera	9/23	Lentils	-36.25	141.11
Longerenong	Vic – Wimmera	9/23	Vetch	-36.63	142.32
Manoora	SA – mid north	8/23	Lucerne	-34.02	138.88
Marrabel	SA – mid north	10/23	Lucerne	-34.12	138.89
Miram	VIC – Wimmera	9/23	Lentils	-36.32	141.33
Moulamein	NSW – Riverina	10/22	Clover	-35.10	144.04
Netherby	Vic – Wimmera	9/23	Lentils	-36.14	141.37
Nhill	Vic – Wimmera	9/23	Lentils	-36.17	141.37
Ninnes	SA – Yorke Peninsula	10/20	Lentils	-33.97	138.05
Pimpinio	Vic – Wimmera	9/23	Lentils	-36.37	142.07
Pompapiel1	Vic – northern	10/21	Lucerne	-36.42	144.13
Pompapiel2	Vic – northern	5/23	Lucerne	-36.42	144.13
Rochester	Vic – northern	10/23	Lucerne	-36.42	144.62
Spalding	SA – mid north	10/23	Lucerne	-33.54	138.62
Susceptible	WA – Central	1999	Lucerne	-31.63	117.72
Tatyoon	VIC – southwest	9/23	Clover	-37.46	143.04
Temora†	NSW – Riverina	6/20	Lucerne	-34.56	147.60
Tintinara	SA – upper southeast	8/22	Lucerne seed	-35.95	140.14
Wanbi	SA – Murraylands	9/20	Lucerne	-34.78	140.33
Willalooka	SA – upper southeast	12/20	Lucerne seed	-36.46	140.33
Yanac	Vic – Wimmera	9/23	Lentils	-36.13	141.31

† Known insecticide resistant populations used in the baseline sensitivity bioassays.

Resistance surveillance bioassays

We screened all field-collected populations for resistance to three insecticide modes of action groups: organophosphate (chlorpyrifos), carbamate (pirimicarb) and synthetic pyrethroids (alpha-cypermethrin) (Table 2). All resistance surveillance bioassays were run using a leaf-dip method as described in Chirgwin et al. 2024. We undertook bioassays in cohorts of four to seven populations over eight rounds, with each round including three individual bioassays, ultimately assessing more than 60,000 aphids in total. Each BGA population was subjected to six to eight concentrations of each insecticide, ranging from 0.00001 to 10 times the field rate, including a water control (Table 2). In each biassay, individual lucerne leaves were submerged in the insecticide solution made up for each concentration or the water control for 5 seconds and then placed on 10g/L of agar within 55mm petri dishes. We prepared six replicate dishes per concentration and transferred 10 3–4-day-old aphids into each dish. Aphids were maintained in a CT cabinet at 18°C with a L16:D8h photoperiod. Mortality was assessed after 72h, aphids were scored as alive (vibrant and moving freely), dead (not moving over a 5-second period) or incapacitated (inhibited movement) for all insecticides.

Table 2. Details of the insecticides used in bioassays.

Insecticide	MoA	Bioassays type	Supplier	Product name	Field rate (mg a.i./L)
Chlorpyrifos	1B	Surveillance	Corteva Agriscience	Lorsban 500EC	1000
Pirimicarb	1A	Surveillance	ADAMA Australia	Aphidex 800 WG	280
Alpha-cypermethrin†	3A	Surveillance	NuFarm Australia	Astound Duo	125
Alpha-cypermethrin§	3A	Surveillance	ADAMA Australia	Alpha-Scud 300SC	120
Flupyradifurone	4D	Baseline test	Bayer CropScience Australia	Sivanto SL	1500
Sulfoxaflor	4C	Baseline test	Corteva Agriscience	Transform WG	240
Flonicamid	29	Baseline test	Ishihara Sangyo Kaisha Ltd	MainMan 500WG	500

† Used in bioassay rounds 1-6; § used in bioassay rounds 7-8.

Baseline sensitivity bioassays

For baseline sensitivity bioassays, we examined two BGA populations (Kieth & Temora) known to be resistant to organophosphates, carbamates and pyrethroids along with the known susceptible BGA population (Table 1) to flupyradifurone, flonicamid and sulfoxaflor.

For flupyradifurone and flonicamid, we used the leaf-dip method as described above. We tested six to eight concentrations from 0.0001× to 100× the registered field rate for use against BGA or other aphid species along with a water control. Mortality was assessed at 72h post exposure for flupyradifurone and 144h for flonicamid.

For the sulfoxaflor bioassay, a micro-topical bioassay was undertaken following the method as described in Ward et al. (2024). Seven concentrations, ranging from 0.0000048 to 48ng of sulfoxaflor 240g/L per aphid were prepared in acetone and tested along with an acetone control. Using a fine-haired paintbrush, ~10–12 adults from the bulk of each population were placed on the abaxial surface of individual lucerne leaves sitting on 10g/L agar in 35mm petri dishes. Seven to 10 replicate dishes were prepared per concentration. After aphid introduction, each petri dish was inverted onto a lid containing a 25mm diameter filter paper. All petri dishes were then placed overnight into a CT cabinet held at 16°C ± 2°C with a L16:D8h photoperiod to allow the aphids to settle before being dosed individually. Sulfoxaflor concentrations were applied under a microscope to ensure accurate placement of droplets using a repeating dispenser with a 10µl syringe to deposit a 0.2µl droplet directly onto the prothorax of each individual aphid. Petri dishes were then inverted and placed into a CT cabinet held at 18°C ± 2°C with a L16:D8 h photoperiod. Mortality was assessed at 48h.

Data analysis

Before analysing and visualising bioassay data, aphid mortality was assessed as the combined numbers of both incapacitated and deceased individuals, since incapacitated aphids are likely to perish before contributing to the next generation.

We used binomial logistic regression to model aphid mortality in insecticide bioassays, which effectively handles binomial data (dead/alive) (Bolker et al. 2009, McElreath 2020). In each model, insecticide concentration and BGA population were fixed-effect predictors. Additionally, a random effect predictor was used at the observation level, assigning a unique random effect to each data point to account for additional variation, preventing model overdispersion (Elston et al. 2001, Harrison 2014). For each model, we first evaluated overall mortality differences among populations (i.e. model intercept) by analysing changes in model deviance using $\chi 2$ tests. When significant differences arose among populations, we performed a post-hoc planned contrasts of means using z-tests to examine pairwise population differences between the known susceptible population and each field population. Next, we assessed whether populations showed differences in mortality based on insecticide concentration (i.e. regression slope differences). We then employed the full model (including both additive and interactive predictors) to estimate the concentrations resulting in 50% mortality (lethal concentration, LC) with 95% confidence intervals (CIs). Resistance ratios were calculated for each insecticide by dividing the LC_{50} values of the field-collected populations by those of the susceptible population. All analyses were conducted using R version 4.4.1 (R Core Team 2024).

Objective 2: Improve baseline biocontrol options for BGA

We aimed to improve the baseline understanding of BGA biocontrol options in two ways. Firstly, we gathered baseline data on what species of parasitoid wasps attack BGA in Australia by undertaking field surveys and laboratory experiments. Secondly, we used sticky trap surveys to obtain baseline data on the generalist aphid predators within lucerne seed paddocks.

Parasitoid field surveys

We assessed each field BGA population collected during the resistance surveillance program for parasitoid wasps. To do this, we monitored each aphid population for evidence of parasitism in the laboratory by placing aphids from each population on lucerne leaves that had been placed on 10g/L agar in 55mm petri dishes. These petri dishes were then held in a CT room at 20°C under a L14:D10h photoperiod for two weeks (which is the timeframe for parasitoids to develop inside an aphid host) and monitored for emergence of parasitoids. Each parasitoid that emerged from the aphids was placed in 100% ethanol and stored at -20°C until they were morphologically identified to the species level.

Parasitoid lab experiments

Only one parasitoid species (*Aphidius ervi*) was found to parasitise BGA from our field survey populations (see Results section for details). We therefore conducted two laboratory experiments to investigate the efficacy of *A. ervi* in controlling other aphid pests of pasture seed crops. The other aphid species tested included cowpea aphids (*Aphis craccivora*), pea aphids (*Acyrthosiphon pisum*), and spotted alfalfa aphids (*Therioaphis trifolli*), which are all known pests of pasture seed crop (Bishop and Milne 1986, Ryalls et al. 2013, Umina et al. 2021b).

To quantify parasitism rates and preferences, we employed a cup system as the experimental arena, which had been previously developed to quantify aphid-parasitoid dynamics (Fig. 2). In each cup, two lucerne stems were placed inside with a sealed-off base filled with water. A fine mesh cloth secured with an elastic band covered the top of the cup. To provide a food source for adult parasitoids, a 30% honey solution mixed with water was provided through a 5mm cotton wool wick at the top of each cup.

We first assessed the ability of *A. ervi* to parasitise each of these four aphid species by exposing *A. ervi* to a single aphid species population at a time (no-choice experiment). Here, we placed one mated female *A. ervi* into the cup with 15 aphids for each species. Each *A. ervi* was transferred into fresh cups of aphids daily for four days. We established 10 replicates for each aphid species. Next, we tested whether *A. ervi* showed a preference to attack any of the four aphid species by exposing them with a mixed population of all four aphid species (choice experiment). Here, we placed 15 individuals of each of the four aphid species into each cup (60 aphids in total). One newly emerged and mated female *A. ervi* was given two hours to parasitise the aphids in each cup. This two-hour exposure was chosen based on our pilot experiment, suggesting this timeframe was suitable for wasps parasitising but not to reach the maximum parasitism rate. Hence, each wasp would not have had enough time to parasitise all aphids in the cup, allowing us to assess which species they preferred to attack first. Eight replicate cups were set up for this experiment.

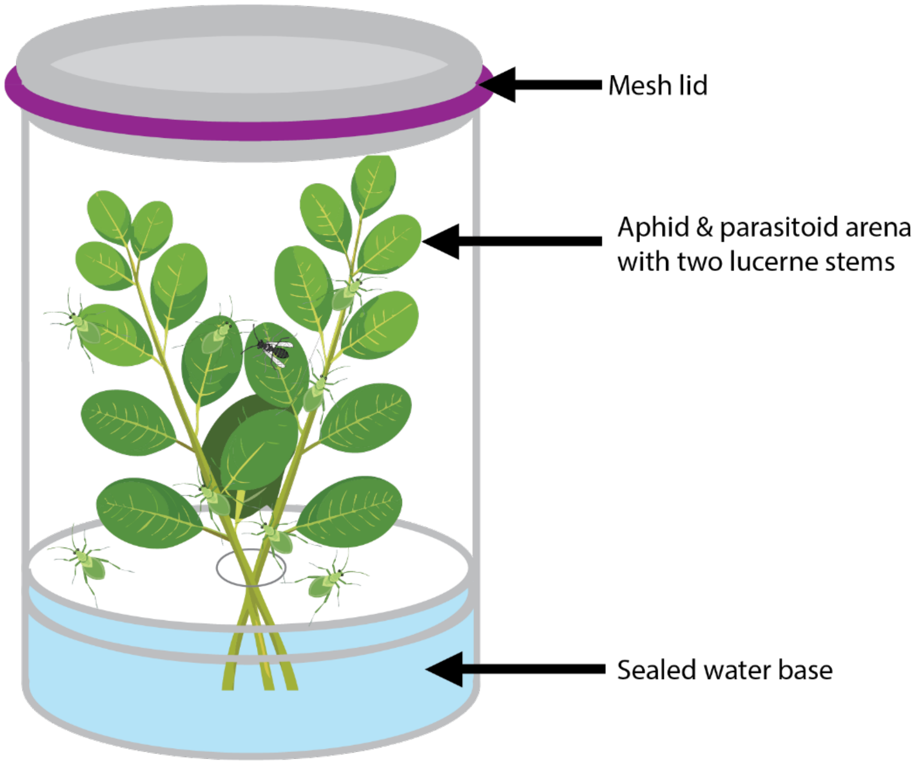


Figure 2. Diagram of the experimental arena to assess aphid-parasitoid dynamics.

After exposure to *A. ervi* across both experiments, aphids were placed into a CT cabinet at 20°C with a L14:D10h photoperiod to allow parasitoid larvae to develop inside aphids. We assessed parasitism rate by visually counting the number of aphid-mummies that developed in the following two weeks. Aphid mummies are easily identified by their swollen appearance and golden colour.

Parasitism rate (%) was calculated as: Number of mummies (N)/total aphids (N). We used a binomial mixed model to assess whether there were differences in the parasitism of aphid host species in both the no-choice and choice experiments. Post-hoc Tukey's HSD tests were undertaken to explore pair-wise differences between aphid species.

Generalist predator surveys

Based on advice and discussions with local agronomists, five lucerne seed paddocks were selected twice per year (in autumn and spring) to deploy sticky traps. Sites that were at the vegetative stage and unlikely to receive any insecticide application during the survey period were selected. Local agronomists assisted with the project by deploying sticky traps. At each paddock, three traps were placed ~20m into the paddock at ~50m apart (Fig. 3). All traps were placed facing the prevailing westerly wind direction and ~20cm above the foliage using a stake. Traps were left for 14 days and then posted to Cesar Australia's laboratory for identification.

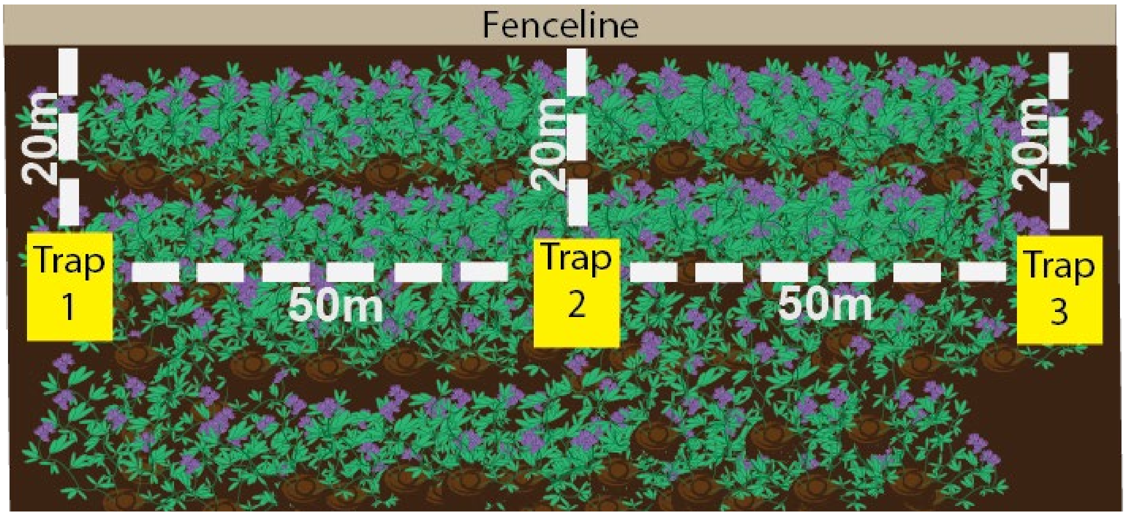


Figure 3. Schematic detailing how sticky traps were deployed at each site.

Invertebrates caught in traps were identified under a microscope, focusing on known predators of aphid species. Identification was to the lowest taxonomic level practical. After assessing the sticky trap data in each sampling period, data visualisation was conducted to assess how the abundance and diversity of generalist natural enemies varied between sites, seasons (spring and autumn) and years.

Objective 3: Communication and extension

Our third objective was to develop communication and extension materials to share our research findings and management guidelines with the pasture and pasture seed industries. To ensure management recommendations balanced scientific rigour with on-farm practicality, an industry advisory group (IAG) was formed, comprising experienced agronomists from the pasture seed industry. The IAG met on five occasions (at six-month intervals) to provide guidance and feedback to Cesar Australia and Lucerne Australia. The agronomists within the IAG included Scott Hutchings, Jess Nottle, James De Barro and Craig Hole. Each member has extensive knowledge of the pasture seed industry and hands-on experience in BGA control. The IAG meeting covered a range of topics including prioritising locations and crop types for resistance surveillance, adoption barriers of previous BGA management recommendations, selecting key communication outputs and platforms, reviewing non-chemical BGA management tools currently in use, providing practical insights into the seasonal challenges growers face with BGA and aphid monitoring techniques to support risk prediction and management. The IAG reviewed and provided input into the final recommendations in this report. We aimed to communicate recommendations across the project via multiple platforms including field-day presentations, webinars, scientific articles, workshops, podcasts and radio.

Results

Objective 1: Insecticide resistance surveillance program

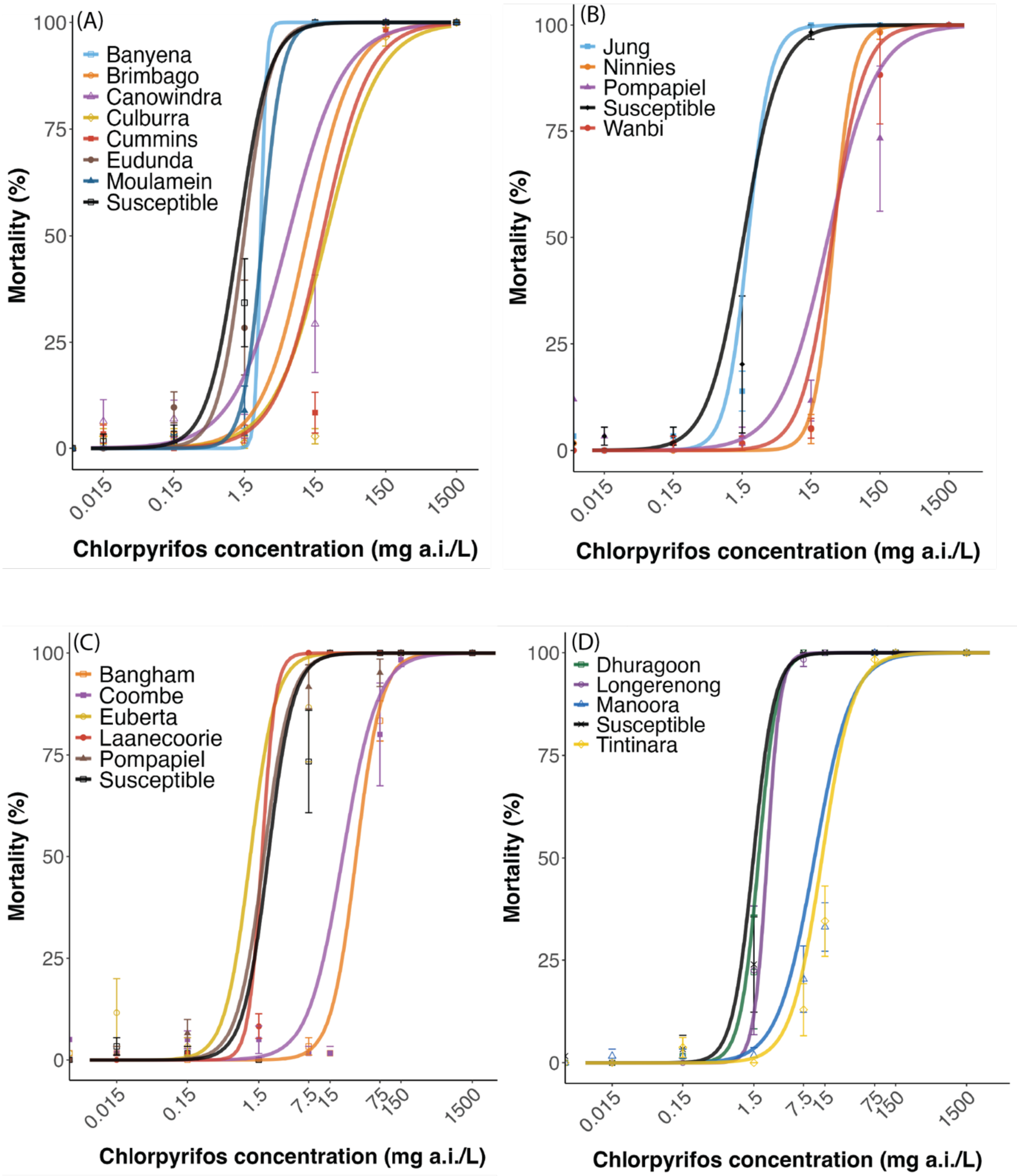
Organophosphates

After 72h exposure, there were significant differences between BGA populations in response to chlorpyrifos in all bioassay rounds: round 1 ($\chi2$ = 36.44, d.f. = 7, *p*<0.01), round 2 ($\chi2$ = 26.41, d.f.= 4, *p*<0.01) round 3 ($\chi2$ = 26.96 d.f. = 5, p<0.01), round 4 ($\chi2$ = 52.14, d.f. = 4, p<0.01), round 5 ($\chi2$ =109.84, d.f. = 5 p<0.01), round 6 ($\chi2$ = 50.75, d.f. = 6, p<0.01), 7 ($\chi2$ = 37.94, d.f. = 4, p<0.01), round 8 ($\chi2$ = 20.47, d.f. = 5, p<0.01). Overall, 20 BGA populations were detected with chlorpyrifos resistance: Brimbago, Culburra, Cummins (Fig.4A), Ninnies, Wanbi, Pompapiel (Fig. 4B), Coombe, Bangham (Fig. 4C), Tintinara, Manoora (Fig. 4D), Lillimur, Coulta (Fig. 4E), Kerang, Pimpinio, Spalding and Marrabel (Fig. 4F), Wilooka, Hinks, Miram (Fig. 4G) and Coolah (Fig. 4H). Resistance ratios ranged from 4 to 25-fold (Table 3).

Table 3. LC_{50} values (and 95% confidence intervals) for BGA populations for responses to chlorpyrifos after 72 h exposure. Populations with significantly higher LC_{50} values than the susceptible population in each round of bioassays are shown with an asterisk.

Bioassay round	Population	LC50 values (mg a.i./L) (± 95% CIs)	Resistance ratio
Round 1	Susceptible	1.21 (0.59–2.50)	-
	Banyena	2.10 (0.89–4.98)	-
	Brimbago*	11.31 (4.24–30.15)	9
	Canowindra	5.99 (2.16–16.60)	-
	Culburra*	20.97 (7.30–60.19)	17
	Cummins*	18.52 (6.99–49.08)	15
	Eudunda	0.93 (0.44–1.95)	-
	Moulamein	1.35 (0.56–3.27)	-
Round 2	Susceptible	1.57 (0.63–3.86)	-
	Jung	1.83 (0.96–3.49)	-
	Ninnies*	31.92 (16.69–61.04)	20
	Wanbi*	30.29 (12.94–70.90)	19
	Pompapiel*	26.39 (8.93–78.00)	17
Round 3	Susceptible	2.03 (0.89–4.66)	-
	Coombe*	22.52 (8.02–63.21)	14
	Laanecoorie	1.66 (0.87–3.15)	-
	Bangham*	34.78 (14.14–85.55)	18
	Pompapiel(autumn23)	1.77 (0.71–4.43)	-
	Euberta	1.14 (0.47–2.73)	-
Round 4	Susceptible	1.48 (0.91–2.39)	-
	Tintinara*	13.60 (8.21–22.54)	9
	Manoora*	10.70 (5.93–19.33)	7
	Dhuragoon	1.75 (1.09–2.80)	-
	Longerenong1	1.48 (0.91-2.39)	-
Round 5	Susceptible	2.48 (1.51–4.08)	-
	Albany	3.33 (1.80–6.14)	-
	Crowlands	2.91 (1.85–4.56)	-
	Lillimur*	60.89 (34.93–106.15)	25

	Coulta*	60.41 (34.12–106.94)	24
	Nhill	4.02 (2.37–6.82)	-
Round 6	Susceptible	3.27 (2.34–4.57)	-
	Kerang*	13.45 (7.99–22.63)	4
	Pimpinio*	19.78 (12–32.58)	6
	Spalding*	15.44 (8.41–28.35)	5
	Kaniva	3.07 (2.28–4.16)	-
	Yanac	2.77 (1.78–4.33)	-
	Marrabel*	30.28 (22.07–41.56)	9
Round 7	Susceptible	1.27 (0.68–2.39)	
	Wilooka*	15.66 (8.92–27.5)	12
	Hinks*	19.6 (9.17–41.93)	15
	Miram*	13.97 (5.3–36.87)	11
	Netherby	2.12 (1.15–3.92)	-
Round 8	Susceptible	3.03 (2–4.58)	-
	Rochester	3.1 (2.25–4.27)	-
	Avalon	1.99 (1.04–3.82)	-
	Kapinnie	2.33 (1.32–4.09)	-
	Tatyoon	2.02 (1.1–3.72)	-
	Coolah*	14.05 (6.91–28.59)	5


(A)
Banyena
Brimbago
Canowindra
Culburra
Cummins
Eudunda
Moulamein
Susceptible
Mortality (%)
Chlorpyrifos concentration (mg a.i./L)
(B)
Jung
Ninnies
Pompapiel
Susceptible
Wanbi
Mortality (%)
Chlorpyrifos concentration (mg a.i./L)
(C)
Bangham
Coombe
Euberta
Laanecoorie
Pompapiel
Susceptible
Mortality (%)
Chlorpyrifos concentration (mg a.i./L)
(D)
Dhuragoon
Longerenong
Manoora
Susceptible
Tintinara
Mortality (%)
Chlorpyrifos concentration (mg a.i./L)

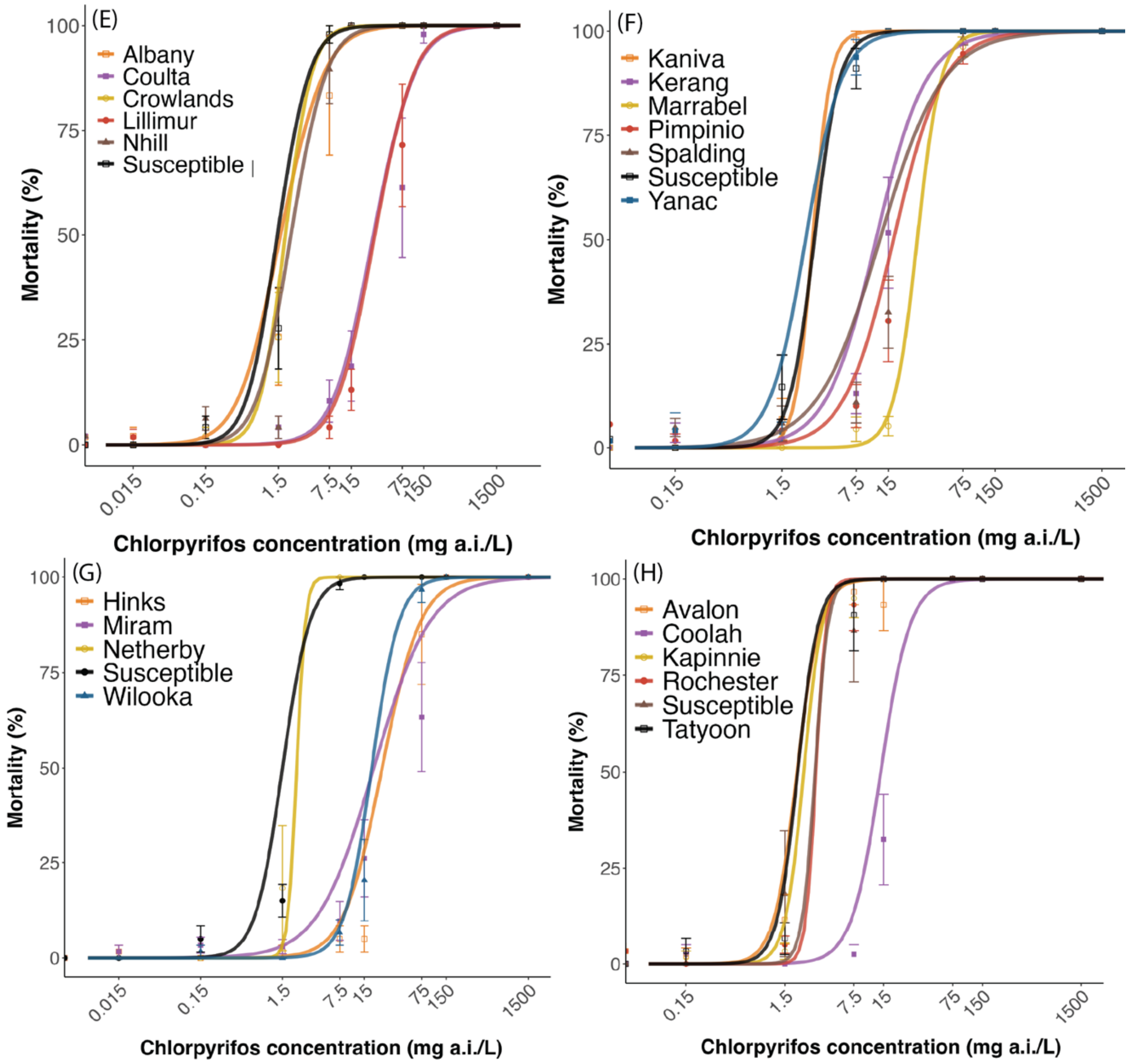


Figure 4. Dose-response curves for 40 field-collected BGA populations (coloured) and a known susceptible population (black) after 72h exposure to chlorpyrifos in eight rounds of bioassays, R1(A), R2(B), R3(C), R4(D), R5(E), R6(F), R7(G), R8(H).

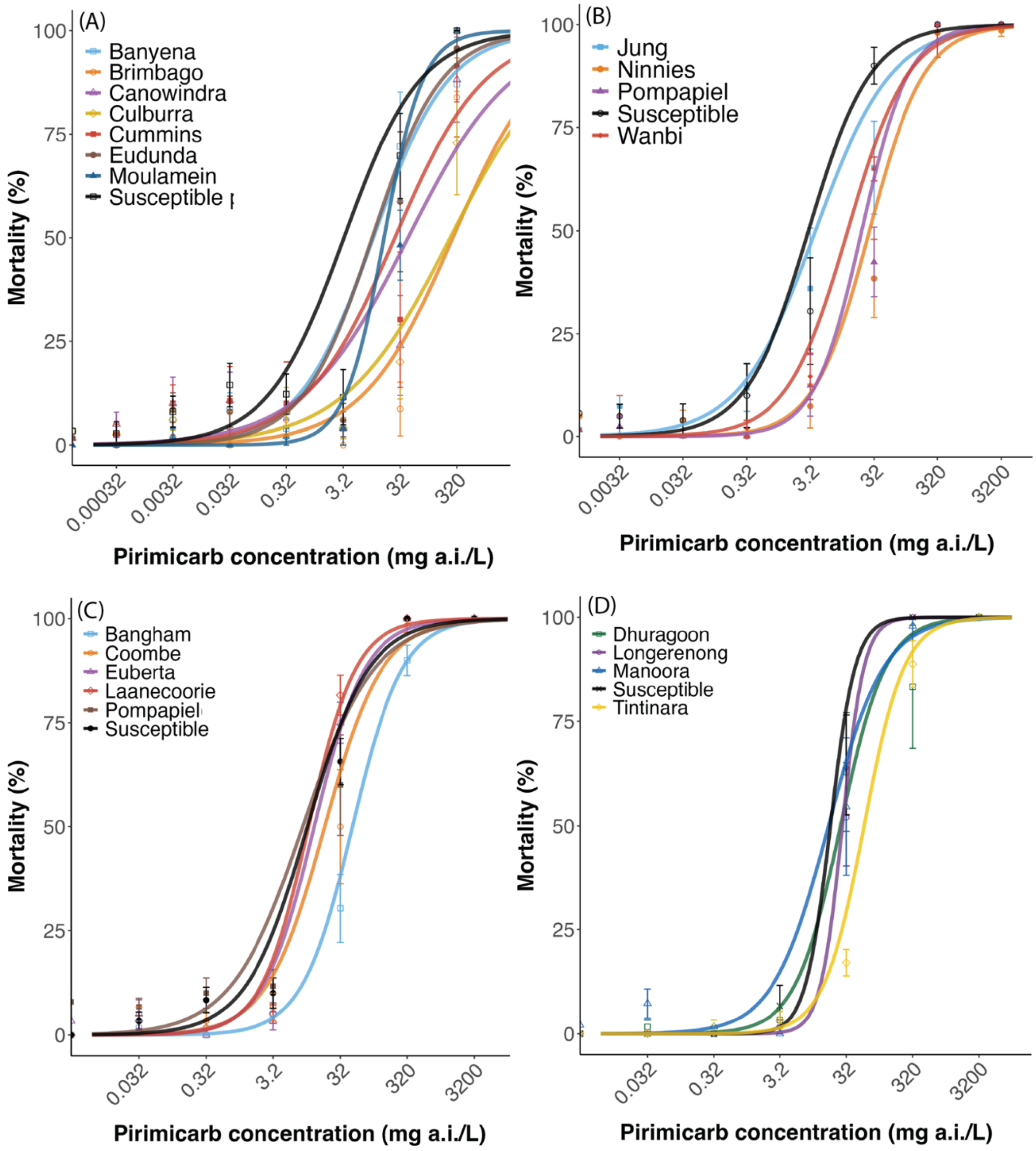
Carbamates

BGA populations showed significant differences in their response to pirimicarb after 72h exposure in bioassay rounds 1–6: round 1 ($\chi2 = 14.29$, d.f. = 7, $p = 0.04$), round 2 ($\chi2 = 17.28$, d.f.= 4, $p < 0.01$), round 3 ($\chi2 = 14.074$, d.f. = 5, p = 0.02), round 4 ($\chi2 = 9.66$, d.f.= 4 p = 0.04), round 5 ($\chi2 = 17.84$, d.f. = 5, p < 0.01), round 6 ($\chi2 = 13.14$, d.f. = 6, p = 0.04). However, no significant population differences were evident in the 7th ($\chi2 = 5.57$, d.f. = 4, p = 0.23) or 8th ($\chi2 = 2.43$, d.f. = 5, p = 0.79) bioassay rounds. In total, 10 populations had significantly higher LC_{50} values than the susceptible population (Table 4): Brimbago, Culburra (Fig. 5A), Ninnies, Pompapiel (Fig. 5B), Bangham (Fig. 5C), Tintinara (Fig. 5D), Lillimur, Coulta (Fig. 5E), Kerang and Pimpinio (Fig. 5F) (Table 4). Several populations including Canowindra, Cummins, Wanbi, Coombe and Hinks showed a decreased in sensitivity to pirimicarb when compared to the susceptible population, however, these were not statistically significant (Table 4). The pirimicarb resistance ratios are lower than that of chlorpyrifos and alpha-cypermethrin, with resistance ratios typically between 4 and 6-fold (Table 4).

Table 4. LC_{50} values (and 95% confidence intervals) for BGA populations for responses to pirimicarb after 72h exposure. Populations with significantly higher LC_{50} values than the susceptible population are shown with an asterisk.

Bioassay round	Population	LC_{50} values (mg a.i./L) (± 95% CIs)	Resistance ratio
Round 1	Susceptible	3.300 (0.73–14.87)	-
	Banyena	10.80 (2.16–54.12)	-
	Brimbago*	270.35 (17.90–4083.20)	82
	Canowindra	44.04 (3.54–548.04)	-
	Culburra*	243.36 (11.92–4967.59)	74
	Cummins	25.82 (3.16–210.43)	-
	Eudunda	10.30 (2.30–46.23)	-
	Moulamein	16.66 (6.46–42.98)	-
Round 2	Susceptible	3.00 (1.37–6.59)	-
	Jung	3.87 (1.61–9.30)	-
	Ninnies*	27.11 (12.84557.22)	9
	Wanbi	12.35 (5.55–27.50)	-
	Pompapiel *	20.51 (10.03–41.95)	7
Round 3	Susceptible	6.55 (2.67–16.06)	-
	Coombe	12.70 (5.22–30.89)	-
	Laanecoorie	8.12 (3.74–17.64)	-
	Bangham*	40.94 (18.84–88.96)	6
	Pompapiel(autumn23)	6.12 (2.50–15.03)	-
	Euberta	12.32 (6.15–24.69)	-
Round 4	Susceptible	21.05 (11.40–38.86)	-
	Tintinara*	74.48 (31.99–173.38)	4
	Manoora	23.43 (7.96–68.93)	-
	Dhuragoon	34.01 (13.08–88.42)	-
	Longerenong1	27.93 (16.37–47.65)	-
Round 5	Susceptible	11.11 (5.54–22.27)	-
	Albany	18.91 (8.11–44.06)	-
	Crowlands	23.01 (11.43–46.34)	-
	Lillimur*	39.11 (17.28–88.5)	4
	Coulta*	36.86 (16.99–79.97)	4
	Nhill	12.55 (6.1–25.81)	-
Round 6	Susceptible	8.58 (4.01–18.37)	-
	Kerang*	37.75 (20.46–69.62)	4
	Pimpinio*	48.20 (25.45–91.29)	6
	Spalding	10.93 (4.10–29.17)	-
	Kaniva	10.37 (4.07–26.41)	-
	Yanac	17.19 (8.34–35.42)	-
	Marrabel	12.99 (5.59–30.16)	-
Round 7	Susceptible	37.45 (19.24–72.89)	-
	Wilooka	26.08 (11.93–57)	-
	Hinks	56.74 (26.95–119.43)	-
	Miram	52.47 (32.24–85.4)	-
	Netherby	26.36 (13.39–51.88)	-
Round 8	Susceptible	20.35 (10.35–40)	-
	Rochester	13.96 (5.69–34.2)	-

Avalon	19.68 (9.4–41.2)	-
Kapinnie	25.77 (14.34–46.33)	-
Tatyoon	14.68 (6.09–35.36)	-
Coolah	26.99 (9.94–73.32)	-



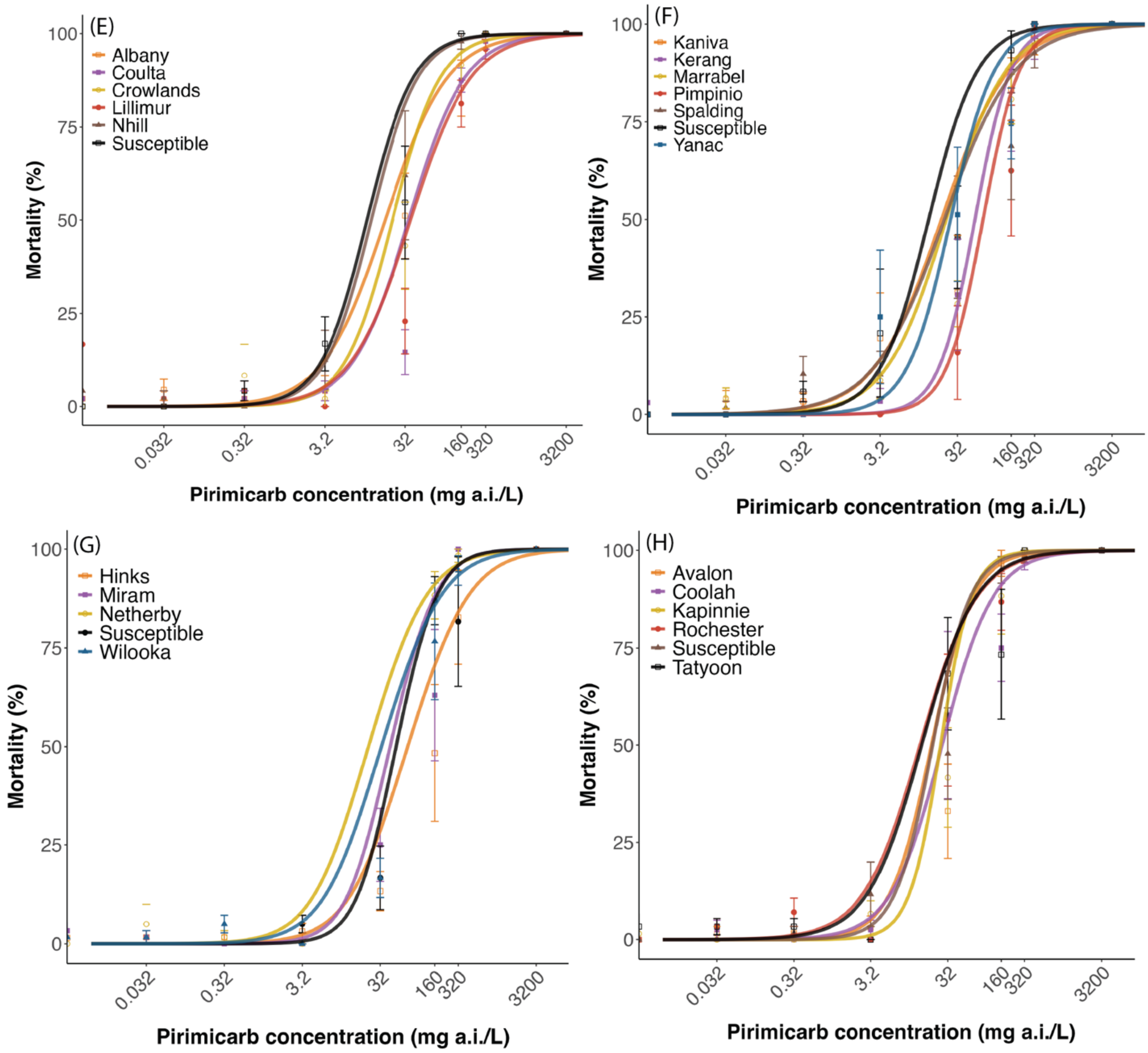


Figure 5. Dose-response curves for 40 field-collected BGA populations (coloured) and a known susceptible population (black) after 72 h exposure to pirimicarb in eight rounds of bioassays, R1(A), R2(B), R3(C), R4(D), R5(E), R6(F), R7(G), R8(H).

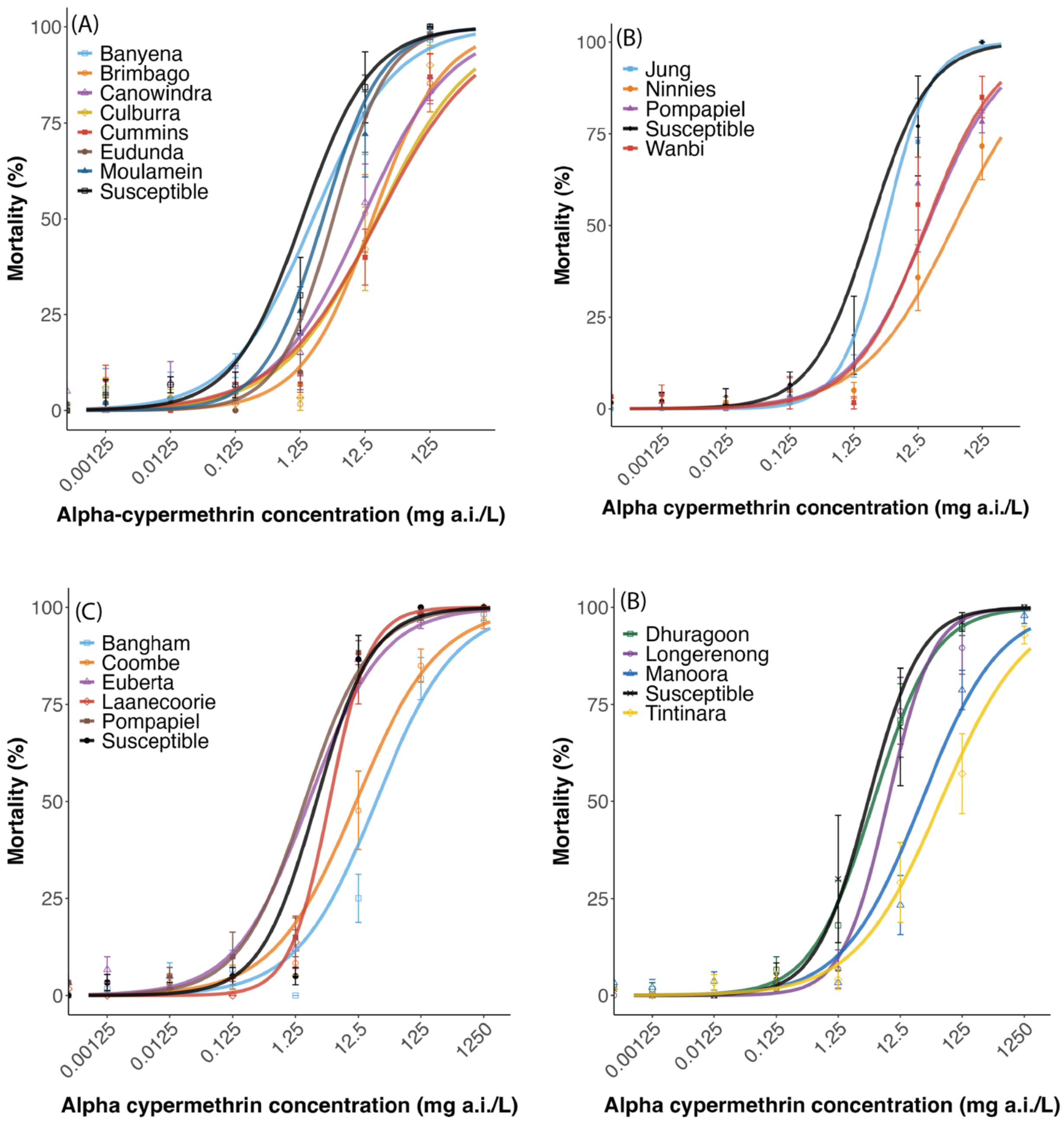
Synthetic pyrethroids

BGA populations showed significant differences in their responses to alpha-cypermethrin after 72h exposure in each bioassay round: round 1 ($\chi2 = 33.31$, d.f. = 7, $p < 0.01$), round 2 ($\chi2 = 19.83$, d.f.= 4 , $p < 0.01$), round 3 ($\chi2 = 26.398$, d.f. = 5, $p < 0.01$), round 4 ($\chi2 = 34.363$, d.f. = 4, $p<0.01$), round 5 ($\chi2 = 18.09$, d.f. = 5, $p < 0.01$), round 6 ($\chi2 = 31.759$, d.f.= 6 $p < 0.01$), round 7 ($\chi2 = 30.503$, d.f.= 4, $p < 0.01$) and round 8 ($\chi2 = 16.846$, d.f. = 5, $p < 0.01$). Twenty populations showed significantly higher LC_{50} values when compared to the susceptible population, indicating resistance to alpha-cypermethrin (Table 5). These populations included Canowindra, Culburra, Brimbago, Cummins (Fig. 6A), Ninnies, Wanbi, Pompapiel1 (Fig. 6B), Coombe, Bangham (Fig. 6C), Tintinara, Manoora (Fig. 6D), Coulta (Fig. 6E), Kerang, Pimpinio, Spalding, Marrabel (Fig. 6F), Wilooka, Hinks, Miram (Fig. 6G) and Coolah (Fig. 6H). Resistance ratios varied between 4 and 19-fold (Table 5).

Table 5. LC_{50} values (and 95% confidence intervals) for BGA populations for responses to alpha-cypermethrin after 72 h exposure. Populations with significantly higher LC_{50} values than the susceptible population are shown with an asterisk.

Bioassay	Population	LC_{50} values (mg a.i./L) (± 95% CIs)	Resistance ratio
Round 1	Susceptible	1.34 (0.63–2.84)	-
	Canowindra*	11.29 (4.16–30.63)	8
	Culburra*	18.97 (5.77–62.40)	14
	Eudunda	4.02 (2.04–7.91)	-
	Banyena	1.97 (0.83–4.65)	-
	Moulamein	2.78 (1.41–5.51)	-
	Brimbago*	16.24 (6.70–39.39)	12
	Cummins*	19.81 (5.72–68.51)	15
Round 2	Susceptible	2.27 (1.06–4.87)	-
	Jung	3.85 (2.04–7.27)	-
	Ninnies*	45.44 (11.21–184.26)	20
	Wanbi*	16.74 (6.15–45.56)	7
	Pompapiel1*	17.513 (6.23–49.24)	7
	Susceptible	2.68 (1.31–5.51)	-
Round 3	Susceptible	2.68 (1.31–5.51)	-
	Coombe*	11.56 (4.77–28.03)	4
	Laanecoorie	4.33 (2.43–7.74)	-
	Bangham*	24.04 (9.52–60.7)	9
	Pompapiel2	1.76 (0.83–3.75)	-
	Euberta	2.04 (0.90–4.61)	-
Round 4	Susceptible	5.50 (2.68–11.30)	-
	Tintinara*	104.18 (33.95–319.69)	19
	Manoora*	48.19 (17.57–132.18)	9
	Dhuragoon	7.21 (3.24–16.03)	-
	Longerenong1	10.86 (5.77–20.46)	-
Round 5	Susceptible	3.65 (1.45–9.17)	-
	Albany	2.92 (1.20–7.14)	-
	Crowlands	3.06 (1.17–7.97)	-
	Lillimur	6.19 (2.44–15.71)	-
	Coulta*	27.45 (10.04–75.11)	8
	Nhill	4.33 (1.62–11.57)	-
Round 6	Susceptible	0.90 (0.43–1.90)	-
	Kerang*	7.19 (3.23–15.97)	8
	Pimpinio*	6.68 (2.92–15.29)	8
	Spalding*	6.41 (3.13–13.14)	8
	Kaniva	2.03 (0.89–4.62)	-
	Yanac	2.78 (1.51–5.12)	-
	Marrabel*	10.43 (3.68–29.54)	12
Round 7	Susceptible	21.34 (6.92–65.8)	-
	Wilooka*	417.5 (102.68–1697.54)	18

	Hinks*	248.37 (88.91–693.81)	11
	Miram*	430.64 (165.98–1117.28)	20
	Netherby	137.51 (69.37–272.61)	-
Round 8	Susceptible	52.42 (17.8–154.35)	-
	Rochester	87.98 (41.33–187.26)	-
	Avalon	38.6 (12.06–123.54)	-
	Kapinnie	197.34 (81.15–479.89)	-
	Tatyoon	59.04 (26.77–130.22)	-
	Coolah*	365.26 (141.11–945.47)	7



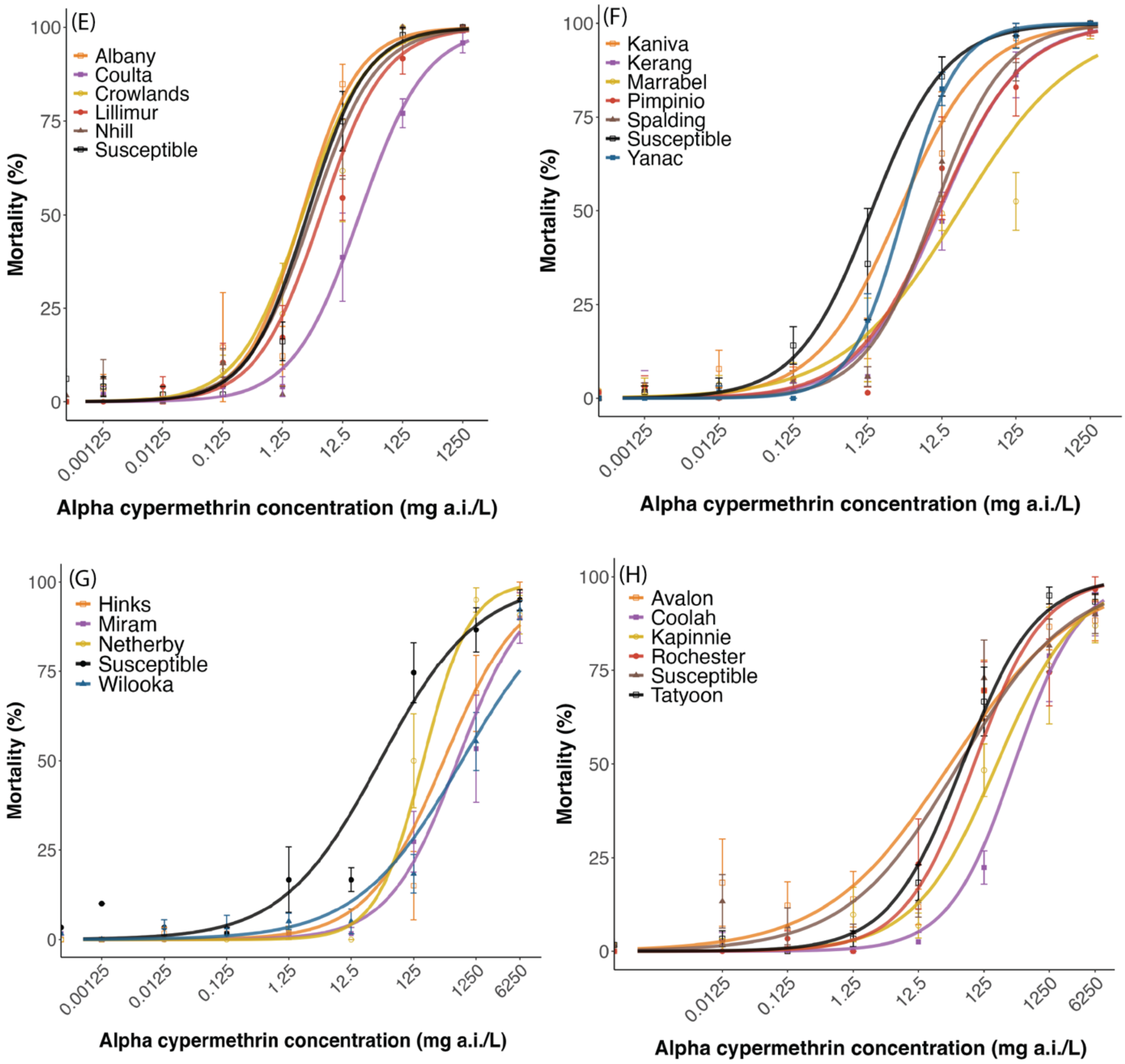


Figure 6. Dose-response curves for 40 field-collected BGA populations (coloured) and a known susceptible population (black) after 72 h exposure to alpha-cypermethrin in eight rounds of bioassays, R1(A), R2(B), R3(C), R4(D), R5(E), R6(F), R7(G), R8(H).

Mapping the distribution of insecticide-resistant BGA across regions and crop types

To date, insecticide-resistant BGA populations have now been identified in 24 locations including 21 from the current project and three from Chirgwin et al. 2022 (Fig. 7). Most resistant populations were found in SA where 16 resistant populations were identified, while five were found in Victoria and three in NSW. Within SA, resistance was discovered in three new regions: Eyre Peninsula, Yorke Peninsula and the mid-north along with new resistant populations being discovered in the upper southeast region where resistance was first discovered in the previous project. In Victoria, resistant BGA populations were found in the Wimmera, Mallee and North Central. In NSW, resistance was detected in the Riverina and Central West. Only one population from WA was evaluated, and no signs of resistance were observed. Resistant populations were collected from various crop types including lucerne seed or pasture (17), lentils (5), vetch (1) and sub-clover (1).

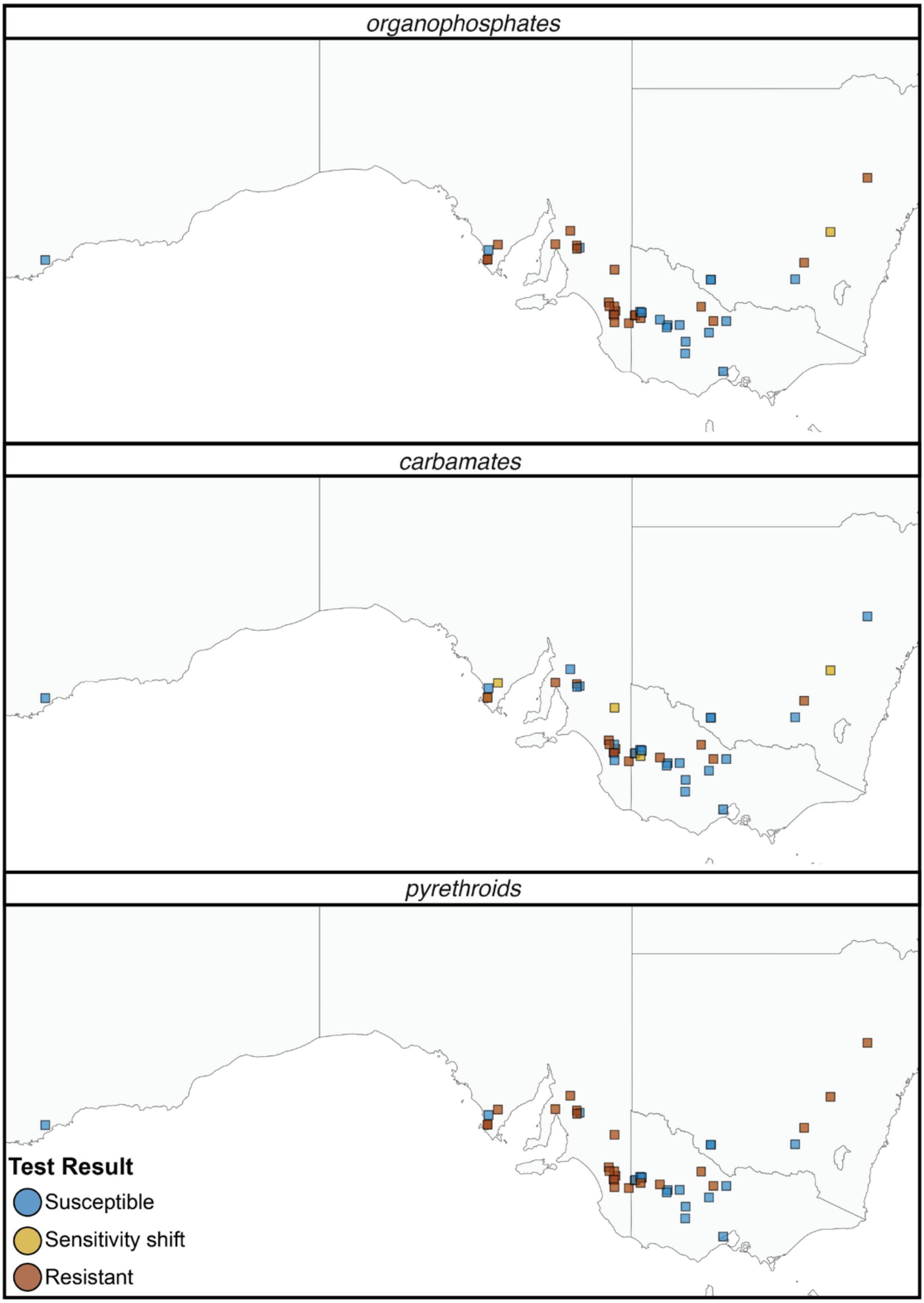


Figure 7. Map of the BGA populations tested and their insecticide-resistance status (carbamates = pirimicarb; organophosphates = chlorpyrifos; pyrethroids = alpha-cypermethrin).

Baseline sensitivity bioassays

No significant population differences were detected in response to flupyradifurone after 72 h exposure ($\chi2 = 3.28$, d.f. = 2, $p = 0.19$; Fig. 7A), sulfoxaflor after 48 h exposure ($\chi2 = 0.70$, d.f. = 2, $p = 0.71$; Fig. 7B), or flonicamid at 144 h ($\chi2 = 5.28$, d.f. = 2, $p = 0.07$; Fig. 7C). For flupyradifurone, LC_{50} values ranged from 65.94 to 201.19 mg a.i/L; for sulfoxaflor, LC_{50} values ranged from 0.03 to 0.05 mg a.i/L (± 0.09) and for flonicamid, the LC_{50} values ranged from 5.15 to 13.61mg a.i./L (Table 6).

Table 6. LC_{50} values (and 95% confidence intervals) and regression coefficients (and standard error) for the three BGA populations for responses to flupyradifurone, sulfoxaflor and flonicamid.

Chemical	Population	LC_{50} values mg a.i./L (95% CIs)	Regression coefficient (±S.E.)
Flupyradifurone	Susceptible	201.19 (89.27-453.43)	0.73 (0.11)
	Keith	65.94 (27.64-157.32)	0.76 (0.10)
	Temora	125.60 (53.39-295.45)	0.71 (0.10)
Sulfoxaflor	Susceptible	0.03 (0.01-0.09)	0.68 (0.08)
	Keith	0.04 (0.02-0.10)	0.72 (0.09)
	Temora	0.05 (0.02-0.12)	0.66 (0.08)
Flonicamid	Susceptible	8.39 (4.45-15.59)	0.68 (0.08)
	Keith	13.61 (8.62-21.47)	1.16 (0.09)
	Temora	5.15 (2.89-9.17)	0.76 (0.09)

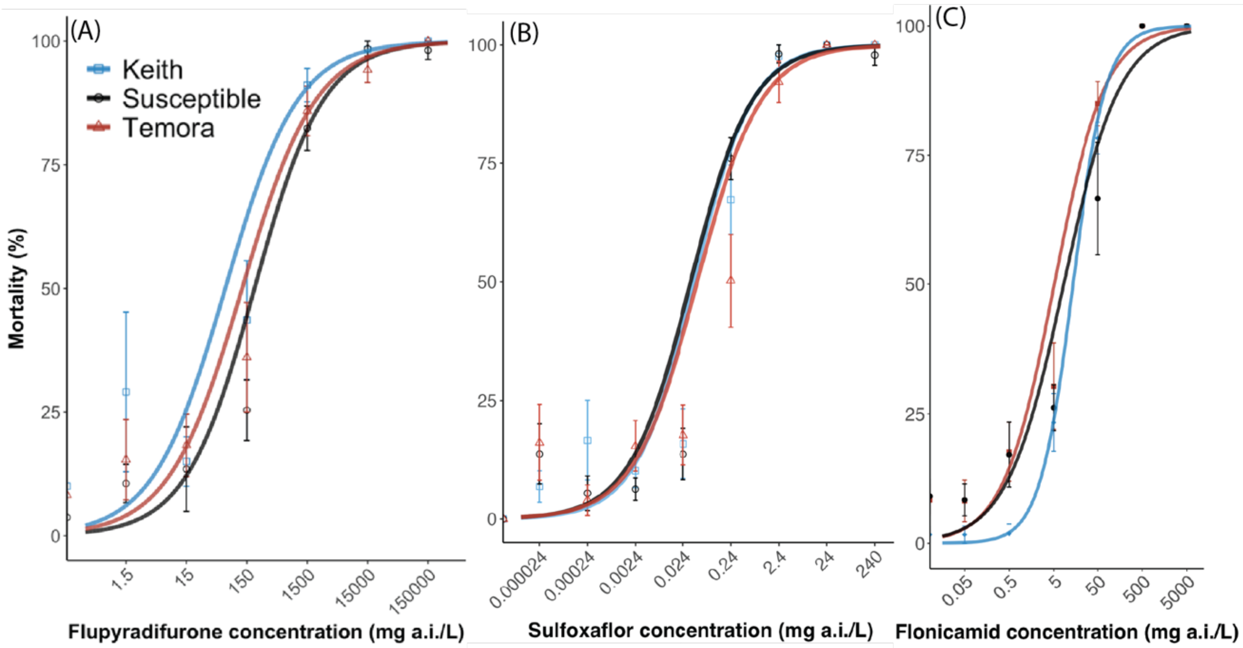


Figure 7. Dose-response curves for two field-collected BGA populations and a known susceptible BGA population in response to (A) flupyradifurone after 72h exposure, (B) sulfoxaflor after 48h exposure and (C) flonicamid at 144h.

Objective 2: Improve baseline biocontrol options for BGA

Parasitoids field surveys

We had 132 parasitoids emerge from BGA that were collected as part of the resistance surveillance. These were from 25 locations in SA, NSW and Victoria and were collected from four different crop types (clover, lucerne, vetch and lentils; Fig. 8). All 132 parasitoids that emerged were the same species: *Aphidius ervi.*

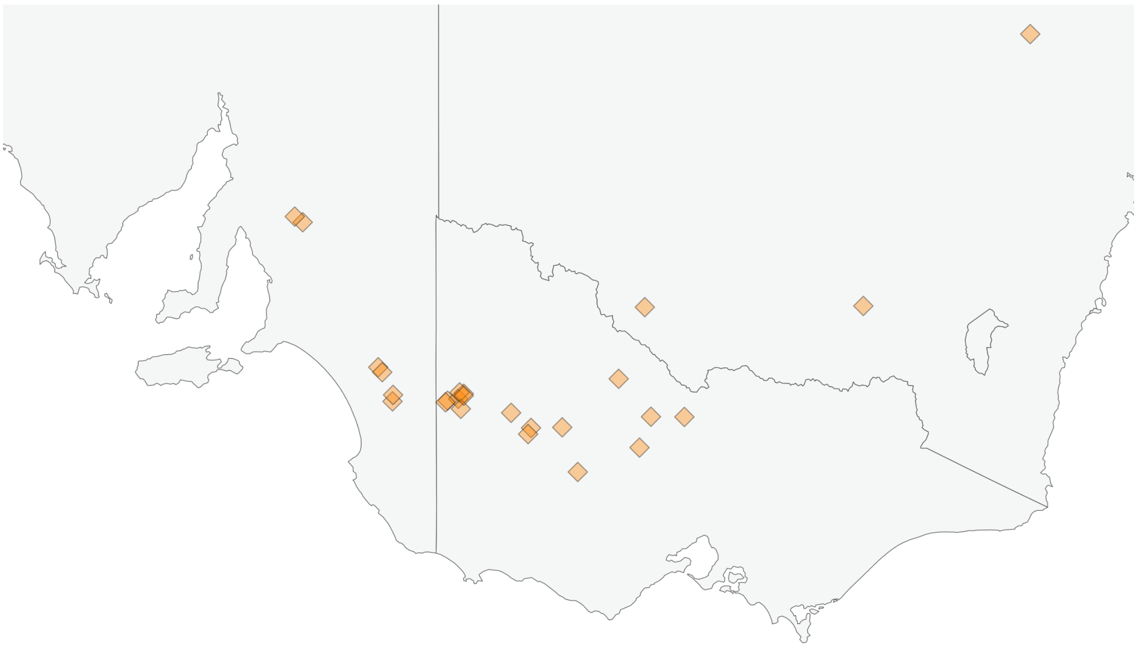


Figure 8. Locations where BGA that had been parasitised by *Aphidius ervi* were collected.

Parasitoids laboratory experiments

Aphidius ervi was capable of parasitising all four aphid species tested here, with the order of preference being BGA, pea aphids, cowpea aphids and spotted alfalfa aphids. However, *A. ervi* showed a significant preference for BGA and pea aphids. In the no-choice experiment, the parasitism rate for BGA and pea aphids was double that of the parasitism rate seen for cowpea aphids and even higher for spotted alfalfa aphids ($\chi2 = 342.65$, d.f. = 3 $p < 0.01$; Fig. 9A). Similarly, when given the choice between the different aphid species, the parasitism rate was significantly higher in BGA and pea aphids compared to the parasitism rate of cowpea aphids and spotted alfalfa aphids ($\chi2 = 20.42$, d.f. = 3 $p < 0.01$; Fig. 9B). A single *A. ervi* was capable of parasitising 70–80% of the BGA and pea aphids in each cup within a day, and showed a reasonable ability to parasitise cowpea aphids, but a poor ability to parasitise spotted alfalfa aphids.

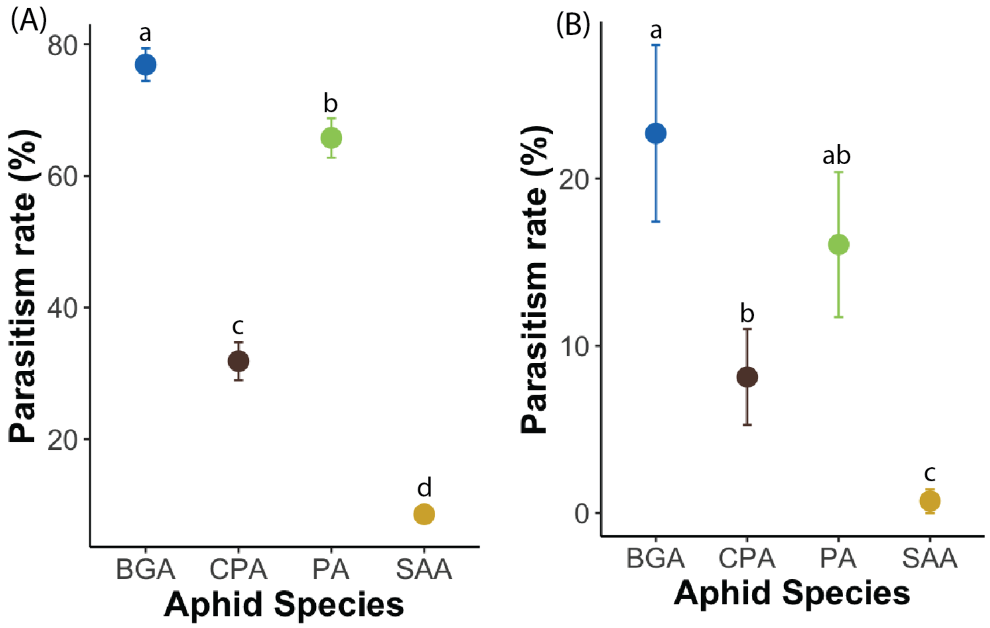


Figure 9: The parasitism rate of *A. ervi* under laboratory conditions. Bluegreen aphids (BGA; *A. kondi*), cowpea aphids (CPA; *A. craccivora*), pea aphids (PA; *A. pisum*) and spotted alfalfa aphids (SAA; *T. trifolii*). Figures show the percentage parasitised by *A. ervi* when (A) provided with a single-species population of aphids for a day and, (B) given a mix of all four aphid species for 2h. Lowercase letters indicate the results of Tukey's HSD test, where points with different letters are significantly different.

Generalist natural enemies via sticky trap surveys

The sticky traps exhibited a greater abundance (Fig. 10) and diversity (Fig. 11) of aphid predators and parasitoids in spring than autumn. On average, aphid predators were ~5 and 25 times more abundant in spring than autumn in 2023 and 2024, respectively. In 2023, parasitoids (*Aphelinidae)* and lacewings (*Hemerobiidae)* were the most abundant natural enemies across the five sites. In 2024, lacewings were rarer, but parasitoids remained at high densities and ladybirds (*Coccinellidae*) became more common. Spiders (*Araneae)*, ladybirds and rove beetles (*Staphylinidae*) were also found at most sites, but at lower abundances. Aphids were present across all sites in 2023, but only five of the eight sites were sampled in spring. Notably, even in the absence of aphids (sites 2 and 3 in spring 2024), we still observed natural enemies in the paddock.

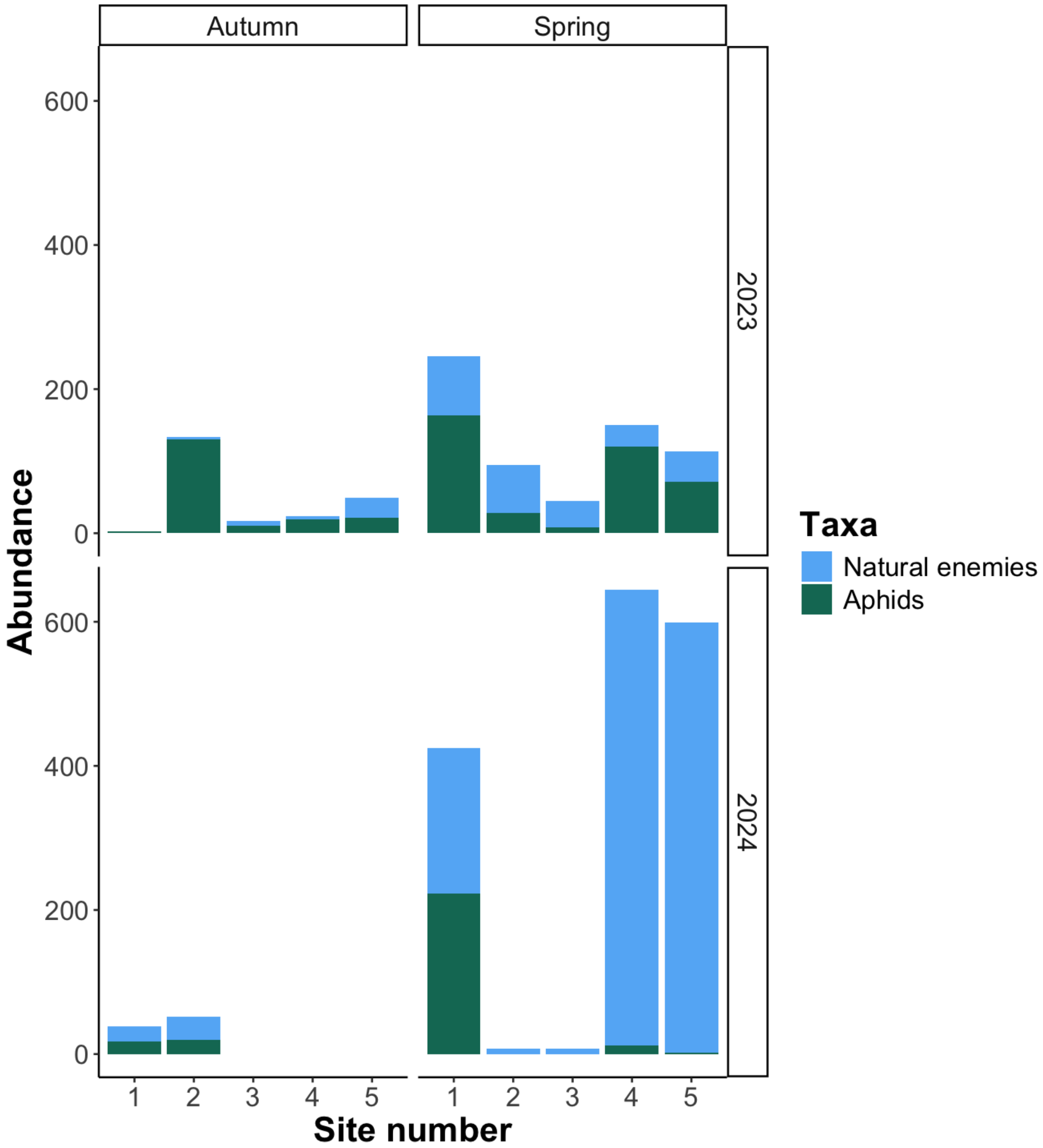


Figure 10. The abundance of aphids and natural enemies caught by sticky traps at each site in autumn and spring 2023. Please note that site 3 in autumn 2024 had no aphids, and the traps at sites 4 and 5 were lost due to environmental damage.

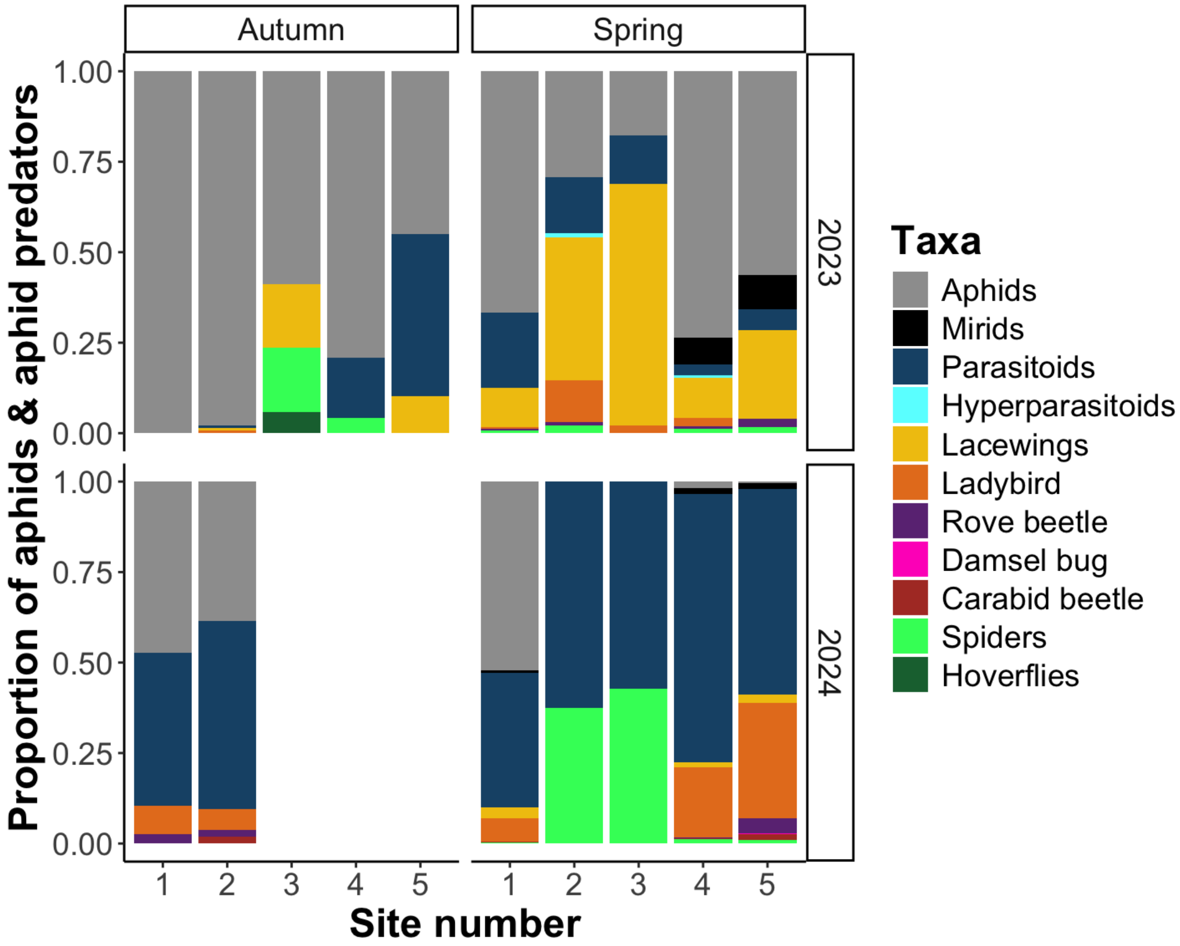


Figure 11. Proportional abundance of aphids and aphid predators caught at each site in autumn and spring in 2023 and 2024. Please note that site 3 in autumn 2024 had no aphids, and the traps at sites 3- 5 were lost due to adverse environmental conditions.

Objective 3: Extension and communication

The project team shared their findings with the industry through various online and in-person events. A comprehensive list of these extension and communication outputs is listed in Table 7. Project findings and recommendations were showcased to the pasture seed industry at the Lucerne Australia Field Day in 2024 and 2025. PestFacts webinars took place in October 2022, May 2023 and September 2024, with the September 2024 session garnering 582 views. The project team enhanced outreach through radio and podcast appearances, catering to listeners who favour interactive or auditory learning. These interactions presented a comprehensive overview of industry and research efforts aimed at tackling resistance challenges. Furthermore, presentations were made at the Crop Protection Forum in December 2023 and November 2024. Additionally, the project's findings and recommendations were conveyed at the GRDC update in Wagga Wagga in February 2025.

During the project, we published several articles both online and in print. The purpose of these articles was to inform growers about the changing distribution of insecticide-resistant BGA and its effects on various crop types alongside alternative (non-chemical) control methods for BGA. Through PestFacts South-Eastern, we emphasised resistance mapping and proposed management recommendations, often achieving positive view counts (e.g. articles from March 2023 and August 2023 each achieved over 400 views). These PestFacts articles were also disseminated to the agricultural community via Cesar Australia's network and various industry partners including Lucerne Australia, Pulse Australia, AgriFutures Australia, GRDC, the Australian Seed Federation and the Grassland Society of Southern Australia. To further engage growers, we extended this outreach with a feature in *The Land* newspaper, several contributions to the Lucerne Leader and in GRDC's GroundCover. This initiative aided in driving sample collections and disseminating findings to a broader audience.

As part of Cesar Australia's broader extension activities, BGA resistance management was incorporated into Cesar Australia's insect identification workshops between 2022–2025 where agronomists and growers received practical training in pest identification and resistance management. We also used Cesar Australia's social media channels to promote research updates and sample submissions, which further expanded our reach.

Several technical resources were also developed to support growers with in-depth, actionable information. The pest profile on BGA, published on AgPest, offered growers a well-organised reference tool for BGA identification and management including interactive maps of where insecticide resistance has been detected. Additionally, a case study published in May 2024 on AgPest provided practical insights into managing BGA in a broad-acre landscape, presenting a real-world example of implementing resistance management strategies.

Table 7: List of extension and communications outputs. A selection of screenshots and images for some of this extension and communication is provided in the appendices.

Output	Type	Engagement
Chirgwin E (September, 2022) 'Help us tackle insecticide resistance in the bluegreen aphid', PestFacts South Eastern	article	125 views
Chirgwin E, Russell J (October, 2022) Insecticide Resistance in Australian Bluegreen Aphids | RESEARCH-IN-ACTION 2022	webinar	142 views
Chirgwin E (April, 2023) Bluegreen Aphids Insecticide Resistance, ACE radio.	interview	
Chirgwin E (April, 2023) Bluegreen Aphids Insecticide Resistance, ABC radio.	interview	
Chirgwin E (April 2023) 'Bluegreen aphid samples sought', The Land.	article	
A tiny but harmful pest: how industry and researchers are tackling bluegreen aphids (April, 2023),,https://agrifuturesonair.buzzsprout.com/1085570/12647292-a-tiny-but-harmful-pest-how-industry-and-researchers-are-tackling-bluegreen-aphids	article	
Jenkins L, Chirgwin E (March, 2023) 'Insecticide resistance in bluegreen aphid spreads into new regions of south-eastern Australia – Cesar Australia', PestFacts South Eastern, https://cesaraustralia.com/pestfacts/insecticide-resistance-bluegreen-aphid-south-eastern-australia/	article	464 views
Jenkins L, Chirgwin E -Insecticide resistance in bluegreen aphid spreads into new regions of south-eastern Australia. Lucerne Leader Issue 68.	article	
PestFacts Webinar | Aphid Update May 2023 (2023), https://www.youtube.com/watch?v=i_abWN9ZHWk2024	webinar	288 views
Veskoukis S, Chirgwin E (August 2023) 'Insecticide-resistant bluegreen aphids found in new crop types', PestFacts south-eastern, https://cesaraustralia.com/pestfacts/new-resistance-in-bluegreen-aphid-parasitoid/	article	435 views
Lowe L, Chirgwin E and Veskoukis S (September 2023) Bluegreen aphid Management this spring: Insecticide-resistant biotypes found in new locations & crop types, Lucerne Leader Issue 70.	article	
Jenkins L (November 2023), 'Bluegreen aphid, Acrythosiphon kondoi', AgPest, https://www.agpest.com.au/pest/bluegreen-aphid	website	
Chirgwin E, (December 2023), 'The emergence of insecticide-resistance bluegreen aphids', Crop Protection Forum 2023' https://www.youtube.com/watch?v=In03nhg3Keg&t=1s	presentation	138 (live) 19 (recorded)
Chirgwin, E, Thia JA, Copping K and Umina PA (2024). Discovery of insecticide resistance in field-collected populations of the aphid pest, Acyrthosiphon kondoi Shinji. Pest Management Science, 80,3,1338-47. https://scijournals.onlinelibrary.wiley.com/doi/full/10.1002/ps.7864	scientific paper	

Chirgwin E and Veskoukis S (March 2024). Insecticide-resistant bluegreen aphids found in new regions and how natural predators can help control them, Lucerne Leader, Issue 72.	article	
Chirgwin E (March 2024), 'Managing the emergence of insecticide-resistant bluegreen aphids', Lucerne Australia Field Day.	presentation	~ 40 (live)
Veskoukis S (May 2024). 'Case study: Tackling insecticide resistant bluegreen aphids in the lucerne seed landscape', AgPest, https://www.agpest.com.au/post/BGA-case-study	article	
Jenkins L, Chirgwin E (September 26, 2024) 'Insecticide resistance update: limited control options for bluegreen aphid in lentils', PestFacts south eastern article https://cesaraustralia.com/pestfacts/insecticide-resistance-update-limited-control-options-for-bluegreen-aphid-in-lentils/	article	96 views
Jenkins L, (September, 2024) 'Managing the emergence of insecticide resistant bluegreen aphid', PestFacts south eastern webinarhttps://www.youtube.com/watch?v=qwzbDnn_PmM&t=1281s	webinar	603 views
Evatt Chirgwin, Sam Ward, Anthony van Rooyen, Lisa Kirkland, Aston Arthur, Alex Slavenko, Karyn Moore, and Paul Umina. Insecticide-resistant aphids in grain crops: where have they spread, and how can we best manage them? Crop Protection Forum 2024	presentation	~ 50 (live)
Evatt Chirgwin, Sam Ward, Anthony van Rooyen, Lisa Kirkland, Aston Arthur, Alex Slavenko, Karyn Moore, and Paul Umina. Insecticide-resistant aphids: where have they spread, and how can we best manage them? GRDC update Wagga Wagga 2025	presentation	~ 100 (live)
Chirgwin E (Feb 2025), Aphid & mite pests of pasture seed: Update on pesticide-resistant varieties spread and tips to manage them, Lucerne Australia Field Day. 2025	presentation	~ 50 (live)
Interactive resistance maps on AgPest website (updated as new populations were detected). https://www.agpest.com.au/resistance-map	Interactive web tool	

Discussion and implications

Objective 1: Insecticide resistance surveillance program

The surveillance program detected insecticide-resistant BGA in several new locations and crop types than previously reported by Chirgwin et al. (2022). In total, 21 new populations of BGA with resistance to organophosphates, carbamates and/or synthetic pyrethroids have been found in southern Australia. The distribution of insecticide-resistant BGA expanded further west (to the Eyre Peninsula), north in New South Wales (near Tamworth) and southeast in several areas of Victoria. This increasing emergence of resistant BGA populations is likely due to strong selection pressures from the use of organophosphates, carbamates and/or synthetic pyrethroids, which promote the evolution of resistant strains over susceptible ones. Reducing this selection pressure poses a challenge for the pasture and pasture seed industries as these three insecticide classes are used for control against several pests of these crops. Consequently, BGA populations are subjected to ongoing selection pressures favouring resistant clones, even when they are not the main target of the insecticide applications. We have no evidence to suggest that resistant BGA have spread beyond SA, NSW and Victoria. However, this cannot be ruled out as we only tested one population from WA and did not test any populations from Queensland or Tasmania in this project.

Insecticide-resistant BGA were more common in some crop types than others. Previously, insecticide-resistant BGA were only found in lucerne seed and pasture crops (Chirgwin et al. 2022), but this project discovered resistant populations in three different crops: lentils, vetch and sub-clover. Notably, insecticide-resistant aphids were found for the first time in key lentil-growing regions including the Yorke Peninsula in SA and the Wimmera and Mallee regions in Victoria. Nevertheless, insecticide-resistant BGA remained most prevalent in lucerne seed and pasture paddocks, particularly in the main lucerne seed production region of south-eastern SA where nearly every population tested was resistant. The perennial cycle of lucerne seed crops may heighten the selection pressures for insecticide resistance to evolve. Lucerne seed crops are usually grown over a period of six or more years, which can allow the same BGA population to persist (even at very low densities) and be subjected to consistent selection pressures over this time. In contrast, other crops that BGA attack (e.g. pulses) are commonly grown in an annual rotation with other crops such as cereals, which BGA cannot survive on. This crop rotation can break up the selection that BGA populations are exposed to over the years. Indeed, crop rotation is a well-established cultural method for managing pesticide resistance in other pests (e.g. weeds) and the limited ability of lucerne seed growers to use this method is a likely driver of the crop-based patterns in insecticide resistance observed here.

BGA exhibited lower resistance to carbamates compared to organophosphates and pyrethroids. Almost all populations showing significant resistance to chlorpyrifos (organophosphates) also exhibited high levels of resistance to alpha-cypermethrin (pyrethroids). However, only about half of these populations demonstrated resistance to pirimicarb (carbamates). Additionally, the resistance ratios for pirimicarb were typically lower than observed for chlorpyrifos and alpha-cypermethrin. Although recent research has begun to reveal the mechanisms underlying pirimicarb resistance in BGA (Thia et al. 2024), whether the mechanism involves epigenetic factors that may allow other (e.g. environmental) factors to influence resistance levels remains untested. For example, some aphid species possess epigenetic mechanisms that allow them to generate greater resistance to pirimicarb in response to environmental signals (Silva et al. 2012). Still, based on our results and consultations with agronomists, pirimicarb appears to control BGA when applied under optimal temperature conditions. Pirimicarb acts via three pathways — contact, translaminar (systemic) activity and fumigant effects (Turner 1995). The fumigant effect is most effective between 20°C and 30°C, but its effectiveness can diminish at cooler temperatures which are common in winter and spring. Therefore, some instances of pirimicarb control failures on BGA are likely due to a combination of low-level resistance and suboptimal application temperatures.

The rising prevalence of insecticide-resistant BGA populations raises concerns, but some encouraging trends have surfaced from the surveillance program. The magnitude of resistance shown by BGA appears to have remained stable over time, suggesting that BGA populations have not intensified their resistance to the three tested insecticide groups. Recently, Cesar Australia was involved in a research project that suggests the insecticide-resistant BGA are the same (or very closely related) clonal genotype (Thia et al. 2024). Given that insecticide-susceptible BGA populations were still identified in 2024, the resistant strain characterised by Thia et al. (2024) has not become fully dominant in southern Australia. As a result, organophosphate and pyrethroid chemicals may still be used to manage BGA in some areas of Victoria and NSW as part of a balanced insecticide rotation plan.

We found no evidence of BGA showing resistance to any of the three newer insecticides tested here: flupyradifurone, flonicamid and sulfoxaflor. This is not unexpected, given that flupyradifurone, flonicamid and sulfoxaflor were only introduced into the Australian market in 2013, 2014 and 2016, respectively. This also indicates no evidence of cross-resistance between flupyradifurone, sulfoxaflor or flonicamid and the resistance mechanisms associated with organophosphates, carbamates and synthetic pyrethroids in BGA. The baseline sensitivity data generated here also provides a valuable benchmark for future resistance monitoring of insecticide resistance to these chemicals. Sulfoxaflor was recently registered for use on BGA in lucerne, but not on any other pasture crops. Flonicamid is currently available on a limited emergency use permit for BGA and mirid control in lucerne seed crops. Anecdotal accounts suggest that sulfoxaflor and flonicamid have effectively controlled BGA in the field. Flupyradifurone is used to control BGA in other countries (e.g. USA) but is not currently registered for use on BGA in Australia. Sulfoxaflor and flonicamid may also provide a valuable option for effective integrated pest management (IPM) methods for BGA as these MoAs are known to be less toxic to many beneficial invertebrates that are predators of aphids (e.g. ladybirds and hoverflies) (Mata et al. 2024b).

Objective 2: Improve baseline biocontrol options for BGA

Aphidius ervi appears to be the only parasitoid species that attacks BGA in southern Australia. The lack of diversity in parasitoids targeting BGA is surprising. Previous research has indicated that aphid pests in Australia are commonly attacked by multiple parasitoid species. For instance, Ward et al. (2021) investigated the parasitoids of several aphid species common to Australian grain crops. On average, each aphid species was attacked by four parasitoid species. Given this lack of parasitoid diversity for BGA, management methods — especially chemical use — need to be carefully designed to support *A. ervi* populations. Unfortunately, insecticides currently registered for controlling BGA in pastures and seed crops (including sulfoxaflor) are highly toxic to *A. ervi* (Overton et al. 2021).

Aphidius ervi have several desirable traits for an effective biocontrol agent. *Aphidius ervi* is a generalist parasitoid of multiple aphid species in legume crops including pea aphids (*A. pisum*), cowpea aphid (*A. craccivora*) and green peach aphid (*M. persicae*) (Milne 1999, Velasco-Hernandez et al. 2017, Cascone et al. 2018). However, our pilot work suggests *A. ervi* will preferentially attack BGA over other aphids commonly present in pasture crops. *Aphidius ervi* is also known to use olfactory cues to seek out legume crops, and each female can parasitise over 300 aphids in their brief (2–3 week) lifecycle (Hagvar and Hofsvang 1991).

Aphidius ervi is mass-produced by commercial Australian breeders, which may provide growers the option to boost natural *A. ervi* populations for future BGA management. This process, known as augmentation, includes mass-rearing a natural enemy species via commercial breeders and subsequently releasing these individuals into the fields to artificially increase natural enemy populations (Collier and Van Steenwyk 2004). Augmentation reduces the time lag between pest populations growing to economically damaging levels and natural enemies responding effectively, whether through migration or breeding, to control the pest population density (Eilenberg et al. 2001, Powell and Pickett 2003). However, augmented parasitoids have primarily been used for controlling insect pests within small or protected cropping areas (Ridland et al. 2020). The effectiveness of releasing commercially produced *A. ervi* to enhance biocontrol efforts in pasture seed crops remains to be tested.

The sticky trap surveys provided baseline information on what generalist predators occupy lucerne seed crops that may help control BGA and other pests. While some of these generalist predators, like ladybirds and lacewings, are common to multiple crop types, the detection here is a necessary step for tailoring management tools and recommendations (see recommendations below for more details) to the pasture seed industry. For example, the findings here can now be used to tailor guides and workshops on how to identify adult and larval stages of these species (see recommendations). Furthermore, the baseline data can be utilised to develop more targeted management strategies that encourage these species (e.g. through cultural methods) to migrate into pasture seed paddocks, along with future research steps to maximise the value that these naturally occurring invertebrates can provide to pasture seed growers.

Objective 3: Extension and communication

A comprehensive extension and communication effort has been undertaken as part of this project to share research findings and BGA management guidelines to the pasture and pasture seed industry. Twenty-three extension and communication activities have been delivered through this project with outputs including industry articles, webinars, peer-reviewed manuscripts, presentations, field days and radio interviews (Table 7).

We worked to provide timely extensions in response to newly identified resistance incursions from our research. As fresh cases of resistance surfaced, our goal was to deliver practical, current guidance to affected growers. This included information on each state's chemical regulations, assistance with species identification and general recommendations such as biocontrol strategies and virus risk assessments. A key challenge in conveying resistance management for BGA involved addressing the intricate nature of the cropping systems at play, which encompass lucerne, pulses, medics, clovers and pastures. Each crop type has its own pest-management requirements, seasonal challenges and varying levels of susceptibility to BGA.

An ongoing challenge is encouraging growers to prioritise insecticide-resistance management strategies. Growers face several challenges each season, and recommendations for pest management can clash with other management priorities. As a result, growers may favour broad-spectrum chemicals for their cost-effectiveness and simplicity. This preference can hinder the implementation of targeted resistance-management strategies that require careful chemical rotation over several years. Although our extension outputs have taken these preferences into account, consistent communication is essential to bridge the gap between growers' existing practices and the long-term advantages of resistance-aware strategies.

Recommendations

Our recommendations are divided into two sections: management recommendations and future RD&E recommendations. The management recommendations section aims to provide growers and advisors with effective strategies for managing insecticide-resistant BGA using the most up-to-date resources and information available. The future RD&E recommendations outline the key research priorities to support the sustainable management of BGA alongside other invertebrate pests in the pasture seed industry.

Management recommendations to pasture-seed producers

R1. Monitoring is essential for assessing BGA risk and selecting a control method

Regularly monitoring crops for the presence of BGA and other pests is an essential first step for assessing the risk of BGA. BGA commonly occurs in pasture seed crops during autumn and spring, but the presence of BGA does not necessarily mean they will reach economically damaging levels. Regular crop monitoring helps growers and agronomists determine whether BGA populations are remaining at low densities or whether populations are increasing to a point where management intervention is needed to prevent economic losses. Monitoring should be more frequent during high-risk periods, particularly in spring and autumn when average daily temperatures range between 18–30°C as these conditions will increase BGA development and reproduction rates. Frequent monitoring is also advised if outbreaks have been known to occur within nearby paddocks as the wing (alates) morph can allow aphids to disperse quickly. Additionally, crops should be monitored closely during stages that are more vulnerable to feeding and virus damage such as establishment or periods of drought stress.

Monitoring aphids alongside natural enemies can help growers and agronomists assess how quickly natural enemies respond to BGA outbreaks. Our parasitoid and sticky trap surveys indicated that one parasitoid (*A. ervi),* along with several generalist predators — such as rove beetles, ladybird beetles, spiders and hoverflies — may assist in controlling BGA in pasture seed crops. We therefore recommend that managers assess the presence of natural enemies alongside aphid populations when considering chemical control during BGA or other aphid outbreaks. It's essential to note natural enemy populations initially lag behind pest outbreaks as they require time to migrate or reproduce in the paddock. The first signs of natural enemy activity in response to pests often appear in their larval stages, particularly for species like ladybirds, lacewings and hoverflies. While these larval stages are less conspicuous than adults — being smaller and duller colours — they play an important role in pest control by actively feeding on aphids. We recommend using sweep net surveys, sticky traps or beat sheets when monitoring for generalist natural enemies. For parasitoids, we recommend monitoring for the presence of mummified aphids on plants. Parasitoid populations grow at an exponential rate — each female has a ~2-week generation time and can produce 300 offspring during this time. As such, the pest control offered by *A. ervi* can increase rapidly. Identification guidelines (including for larval stages) and further advice on supporting these natural enemies in crops can be found in Cesar Australia's Beneficial Profiles.

R2. Ensuring the correct identification of aphid species before selecting a control method

Multiple aphid species coexist in pasture seed crops including cowpea aphids, pea aphids, spotted alfalfa aphids, green peach aphids and faba bean aphids. Among them, BGA can easily be confused with pea and spotted alfalfa aphids. Indeed, several samples of pea and/or spotted alfalfa aphids were misidentified and submitted by agronomists assisting in the project's surveillance efforts. These three species display different insecticide resistance profiles. Unlike BGA, pea aphids show no resistance to any insecticides in Australia, while some spotted alfalfa aphid populations have resistance to carbamates and organophosphates (Holtkamp et al. 1992). We recommend growers use the aphid identification resources currently available to ensure they select the correct chemicals to manage aphid pests. [The GRDC Back Pocket Guide on Crop Aphids] and [Cesar Australia's Pest notes] both provide ID guidelines. Furthermore, we encourage growers to attend PestFacts insect ID workshops to gain hands-on experience in identifying different aphid species.

R3. Select insecticides with lower toxicity to natural enemies by using the beneficials toxicity table

While the beneficials toxicity table was tailored to grain crops as part of a GRDC-funded project, this table includes many of the same foliar insecticides used in the pasture and pasture seeds industry. Additionally, the table includes many of the beneficial species found in our sticky trap surveys. The toxicity table has been developed to help growers and advisors make informed choices about the insecticides they use in their crops. A significant challenge is that most chemicals used for aphid control in pasture seed crops such as organophosphates, carbamates, synthetic pyrethroids and sulfoxaflor are highly toxic to parasitoid wasps. However, some insecticides, like flonicamid, appear to be much less toxic to parasitoids. Outside of parasitoids, sulfoxaflor is less toxic than the older MoA to some groups of generalist predators found in our sticky trap surveys including lacewings, rove beetles and ladybirds.

R4. Rotate between insecticide MoAs to reduce the risk of insecticide resistance

The emergence and spread of insecticide resistance in BGA is primarily the result of selection pressure from the widespread use of organophosphates, carbamates and synthetic pyrethroids. To prevent further resistance development, one of the most effective strategies is to reduce these selection pressures by rotating between different MoA groups when insecticide applications for BGA (or other invertebrate pests) are necessary. However, pasture seed growers currently have limited alternative options to rotate between MoAs to control BGA. Sulfoxaflor was recently registered for use on BGA in lucerne and flonicamid is currently under a limited emergency use permit for lucerne seed crops. Therefore, both sulfoxaflor and flonicamid can offer valuable alternatives for lessening resistance selection in lucerne seed paddocks, particularly when carbamates, organophosphates and pyrethroids are required to manage other common pests (e.g. mites and mirids). Unfortunately, outside of lucerne, other pasture seed crops have no other registered foliar insecticide options to facilitate MoA rotation beyond carbamates, organophosphates and pyrethroids.

R5. Select the newer MoAs in regions where BGA commonly shows resistance to older MoAs

Our bioassays here showed sulfoxaflor and flonicamid can provide controls for BGA that are resistant to the three other MoAs, but growers should only use this product at the recommended label rates. Sulfoxaflor has recently been registered in all lucerne crops, and flonicamid is under an emergency-use permit (PER94374) for use in lucerne seed crops until August 2025. Therefore, sulfoxaflor and flonicamid currently provide the most reliable registered MoA option for BGA control in lucerne crops. However, the higher cost of sulfoxaflor and flonicamid relative to the older MoA has (anecdotally) deterred some growers from using these for BGA control or led some growers to apply them below the registered label rates. We strongly advise growers to avoid applying sulfoxaflor or flonicamid (or any other insecticide) at below-the-label rates. Not only does this practice risk poor pest control, but it also increases the likelihood of resistance in the future. While applying below-the-label rates may have some short-term appeal, this practice will lead to more resistance challenges arising in the long term.

R6. Use pirimicarb strategically to control BGA, ensuring it's applied under optimal environmental conditions to maximise effectiveness

While our bioassays suggest many populations exhibit carbamate resistance in southeastern Australia, the resistance level was consistently lower than that of organophosphates and synthetic pyrethroids.

Our bioassay results are consistent with what growers observed in the field as pirimicarb was reported to offer effective BGA control when applied under its optimal temperature range. As discussed earlier, pirimicarb achieves the highest efficacy when applied between 20–30°C due to the fumigant action of this chemical. Thus, growers should be wary that managing BGA with low-level pirimicarb resistance may become more challenging in cooler times of the year when its efficacy will decline. Where possible, growers should wait for a 1–2 day window of warm (>20°C) weather before applying pirimicarb for BGA control. While carbamates currently offer an effective and economical tool, growers must be mindful that overreliance on this single MoA for BGA control will likely drive resistance to greater levels. Repeated application of pirimicarb in the same paddock for BGA and other pests within a single growing season should be avoided whenever possible to help maintain the efficacy of this mode of action.

Some growers have also reported anecdotal success in applying carbamates mixed with paraffinic oil to achieve greater control efficacy. Paraffinic oils can help manage aphids by physically coating them to prevent their feeding and/or death by asphyxiation. However, growers should take care when using paraffinic oil as it may cause phytotoxicity damage to crops if used improperly.

R7. Organophosphates and pyrethroids should be avoided in pasture seed-growing regions of South Australia

BGA populations resistant to organophosphates and synthetic pyrethroids continue to be detected in new regions but appear more common in some regions than others. Our surveillance indicates that BGA with moderate levels of resistance to organophosphates and synthetic pyrethroids is most prevalent in SA, with the majority of populations screened exhibiting resistance. Furthermore, based on anecdotal consultations with affected agronomists in this region, the levels of organophosphate and synthetic pyrethroid resistance appear sufficient to cause control failures. Therefore, we recommend South Australian growers avoid using organophosphates and synthetic pyrethroids for BGA control as there is a high risk of control failure. In Victoria and NSW, resistance to organophosphates and synthetic pyrethroids is increasing, but susceptible populations are still being detected. As such, organophosphates and pyrethroids may still serve as a viable option within a balanced rotation plan in some regions of Victoria and NSW. However, growers should be aware that using these chemicals carries a risk of control failure. To minimise this risk, we recommend they perform a test patch on a small area of their paddock before broader application.

R8. Neonicotinoid seed treatments can help manage aphids in some pasture and lucerne seed crops to protect against early aphid infestations and viruses transmitted by these pests

Seed treatments are particularly beneficial in the early emergence stages when plants are more vulnerable to virus transmission via aphids, thus acting as a crucial protective measure early in the season. However, their effectiveness diminishes as plants grow, and they cease to provide protection later in the season during spring when the numbers of BGA peak and cause harm via direct feeding. Furthermore, seed treatments only protect plants during their first growing season, limiting their utility in perennial crops including lucerne seed. However, seed treatments remain an important tool for protecting subterranean clover from many of the viruses carried by BGA (including bean yellow mosaic virus). Although the status of neonicotinoid resistance in BGA is untested, no control failures have been reported to date.

R9. Selecting crop varieties with a higher tolerance to viruses

BGA can carry several different viruses, and the risk of the virus to crop yield varies greatly across pasture seed crops**.** The risk of the virus tends to be greater in clover and sub-clover crops than in others (e.g. lucerne). Avoiding growing virus-sensitive crops adjacent to perennial lucerne or pasture can decrease the risk of BGA migrating into the crop. By sourcing seeds tested for aphidtransmissible viruses (e.g. alfalfa and cucumber mosaic viruses), growers can prevent the presence of viruses in a paddock. Information on the virus tolerance sub-clover varieties can be found via the NSW DPI.

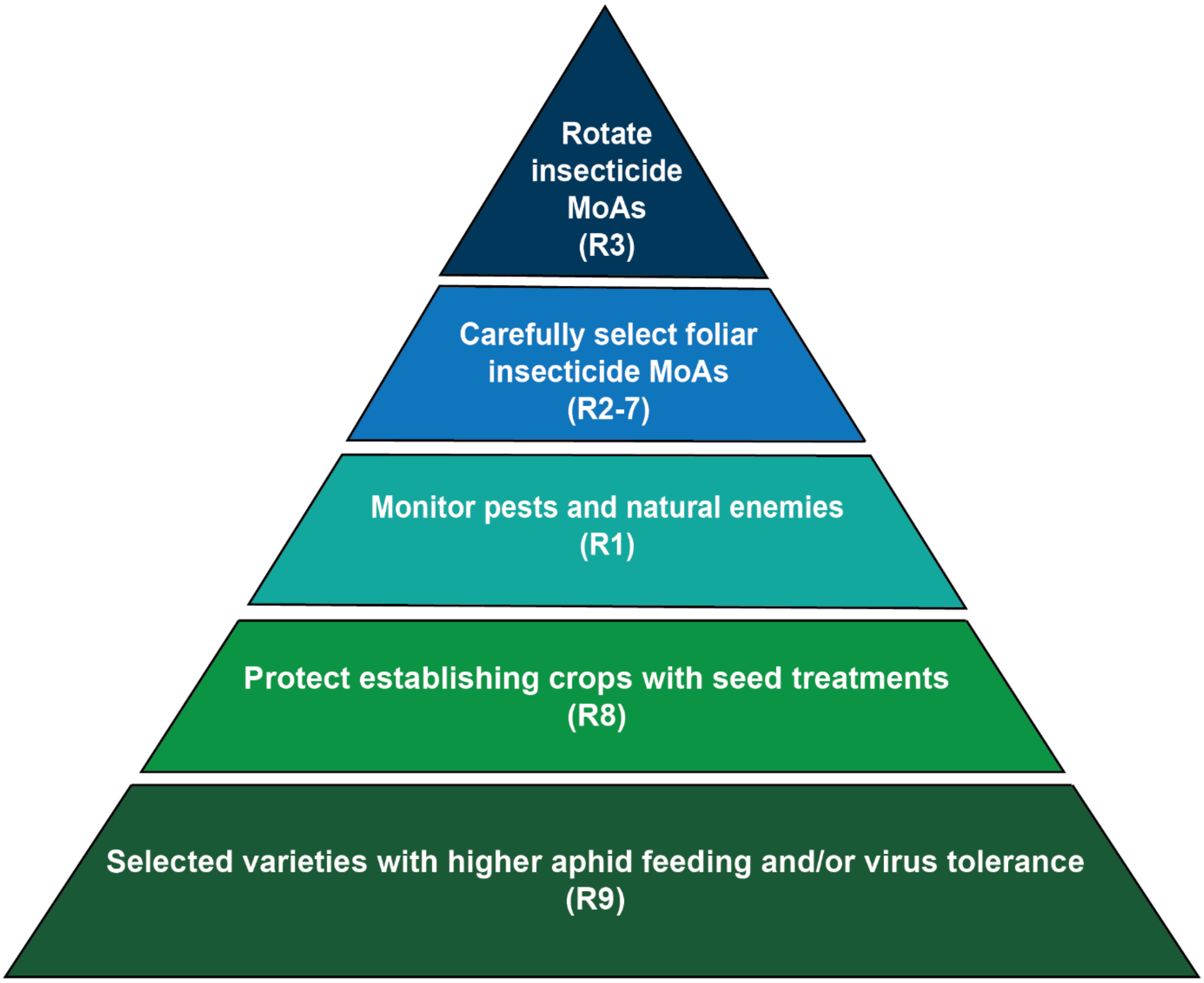


Figure 12. The pyramid provides a tool for how and when growers and agronomists should implement the nine management recommendations (R1-9) listed above. The pyramid starts from a solid base of variety selection and builds upwards to situations where rotations between insecticide MoA are needed to control successive outbreaks of BGA (or other pests) within a season.

Future RD&E recommendations

F1. Develop an insecticide resistance management strategy (IRMS) for pasture seed crops

A consistent challenge raised by growers and agronomists is managing the rotation of insecticide use for BGA while addressing the control needs of other pests. For example, red-legged earth mites (RLEM) are a major pest of pastures in Southern Australia and require foliar insecticide treatment most years. Due to the limited registered options and the evolution of insecticide resistance in RLEM, organophosphates remain the only effective foliar option for RLEM in many regions in South Australia (Mata et al. 2024a). Furthermore, given the perennial nature of many pasture seed crops, seed treatment options are not available as they are for annual crops. Relatedly, other invertebrate pests, particularly cowpea aphids and mirids, are currently managed using the same limited range of insecticides needed to control BGA. While our recommendations above focus on BGA, a broader strategy is necessary to manage the risk of insecticide resistance in BGA alongside other invertebrate pests of pasture seed crops.

IRMSs assist in addressing resistance in existing pest populations, preventing the development of resistance, and aiding in the recovery of insecticide susceptibility in already resistant groups (Umina et al. 2019). Creating effective IRM strategies is crucial for minimising resistance evolution and ensuring long-term efficacy and utility of current insecticide options. IRMSs have been developed for individual pest species such as RLEM that affect the pasture seeds industry. However, a regional and/or crop-specific IRMS offer a more effective option for the pasture seeds industry, and such approaches have successfully seen high adoption rates in other industries (e.g. cotton)(Wilson et al. 2018). Simply put, IRM strategies promote judicious insecticide use through three actions: 1) applying insecticides only when pest levels exceed economic thresholds; 2) minimising the use of broad-spectrum insecticides when feasible and 3) rotating insecticides to prevent the same MoA from being used on consecutive pest generations. To refine steps 1 and 2, additional research and industry collaboration are needed to establish guidelines for what insecticides are most suitable and when they should be applied.

F2. Development of a robust, practical monitoring program

Monitoring is central to the management recommendations listed above, and there remains a huge scope to improve the guidelines and tools used for monitoring. Presently, growers and agronomists use several methods for monitoring pests including visual inspections, sticky traps, sweep nets and pheromone traps. The methods used, along with the frequency of use and confidence in interpreting the results of these for monitoring, vary between growers and agronomists, and are largely based on their own experiences. In turn, developing monitoring guidelines or rules of thumb would be most useful for junior growers and agronomists.

Monitoring guidelines and rules of thumb can help growers and agronomists better assess whether natural enemies are keeping pests in check and when insecticide application becomes warranted. A large obstacle preventing growers and agronomists from choosing biocontrol over insecticides is the uncertainty regarding how quickly biocontrol agents will respond to pest infestations. Addressing this issue is difficult, as predicting the interactions among various pest and predator species presents a complex challenge. However, there is huge potential to enhance current management guidelines through field-based research and collaboration with local agronomists to establish biocontrol rules of thumb.

Establishing an economic threshold for BGA in pasture seed crops would guide growers and agronomists on when a foliar spray becomes the most economically viable pest management option. While economic thresholds have been developed for BGA in other crop types (mainly pulses), no economic thresholds exist for BGA in any pasture seed crops. This creates uncertainty around when an insecticide spray is needed, ultimately making managing the risk of insecticide resistance more challenging. However, we acknowledge that the cost of developing an economic threshold for BGA for individual pasture seed crops is substantial and may be prohibitive at this time. Therefore, we recommend developing improved monitoring methods and rules of thumb to address current needs.

F3. Diversifying the number of insecticide options registered for aphids

New MoAs should be registered for aphid control in pasture and pasture seed crops to facilitate better MoA rotation (see R4). While carbamates can effectively control BGA in most cases, there remains a sizeable risk of BGA developing increased resistance to this MoA. As carbamates remain the only registered foliar options for most pasture seed crop types (other than lucerne), growers lack alternative insecticides with different MoAs required for insecticide rotation. Obtaining additional permits and registrations is by no means a simple process. Registering products can be expensive, time-intensive and subject to various regulatory constraints. Yet, depending on a limited number of insecticides to manage BGA may exacerbate resistance problems. Furthermore, IRMS (see F1) are more likely to succeed if more MoAs are available to facilitate rotation.

F4. Continued cross-industry cooperation

BGA attacks multiple crops, not only those in the pasture and pasture seed industries. Pests that attack multiple crops are prone to developing insecticide resistance (Rane et al. 2016). Ineffective insecticide practices in one or more crop types can quickly lead to resistance emerging and spreading to other crop types. The collaborative efforts of this project with the GRDC have fostered more coordinated and improved outcomes for BGA management. Cross-industry cooperation should be encouraged in any future efforts involving BGA to enhance ongoing management and mitigate its current economic impact on multiple industries.

F5. Monitor the distribution of insecticide-resistant BGA using recently developed molecular tools

Resistant BGA will spread into new regions over time, and ongoing surveillance efforts can assist growers in making more informed management choices. Recently, Thia et al. (2024) identified a strong molecular candidate for the mechanism underlying BGA's insecticide resistance to all three MoA, and this mechanism can now be repurposed as a molecular diagnostic tool. This tool can enable insecticide resistance to be tested faster (in days instead of weeks) and is significantly cheaper than the laboratory bioassay methods previously required. Such diagnostic tools are already used in other pests such as the green peach aphid. In turn, molecular diagnostics can give growers quicker results to make timely management decisions. Such diagnostic methods can be useful for diagnosing whether resistant BGA has spread into new regions (e.g. Northern Tasmania and Western Australia) where pasture seed crops are common. Molecular tools can also help growers and agronomists in the key pasture-growing regions of SA judge whether resistant BGA persists in their paddocks. Aphids are highly dispersive and insecticide-susceptible populations can return to areas where resistance was previously reported. In turn, growers could relatively easily and cheaply keep track of the status of resistant BGA on their property by collecting and posting BGA samples for molecular testing.

F6. Advertising the risk of resistance on products or at chemical selling points

While the project team communicated and extended the research through several pathways (Table 7), there remain avenues for more targeted communication about insecticide resistance. One avenue discussed with the project team and the industry advisory group was developing labels or stickers on products to inform growers and agronomists about which invertebrates have developed insecticide resistance to this MoA. Developing such stickers would require considerable collaboration among multiple groups (CropLife Australia, chemical production companies, local retailers and regulatory bodies). However, this system would provide a simple way for growers to reduce the risk of chemical control failures.

F7. Developing insect identification tools and training courses tailored to the pasture seed industry

Our project provided baseline data on natural enemies that suppress aphids and other pests in pasture seed crops. However, further steps can be taken to ensure growers fully benefit from the natural biocontrol resources available. The first step in improving biocontrol is enabling growers and agronomists to identify species of natural enemies and understand what pests these control. While the online tools listed above offer valuable guidance, in-person training can provide growers and agronomists with hands-on experience applying these ID skills. We recommend that in-person workshops be conducted in key pasture seed-growing regions, where resistance issues are common.

F8. Breeding and testing aphid-tolerant cultivars

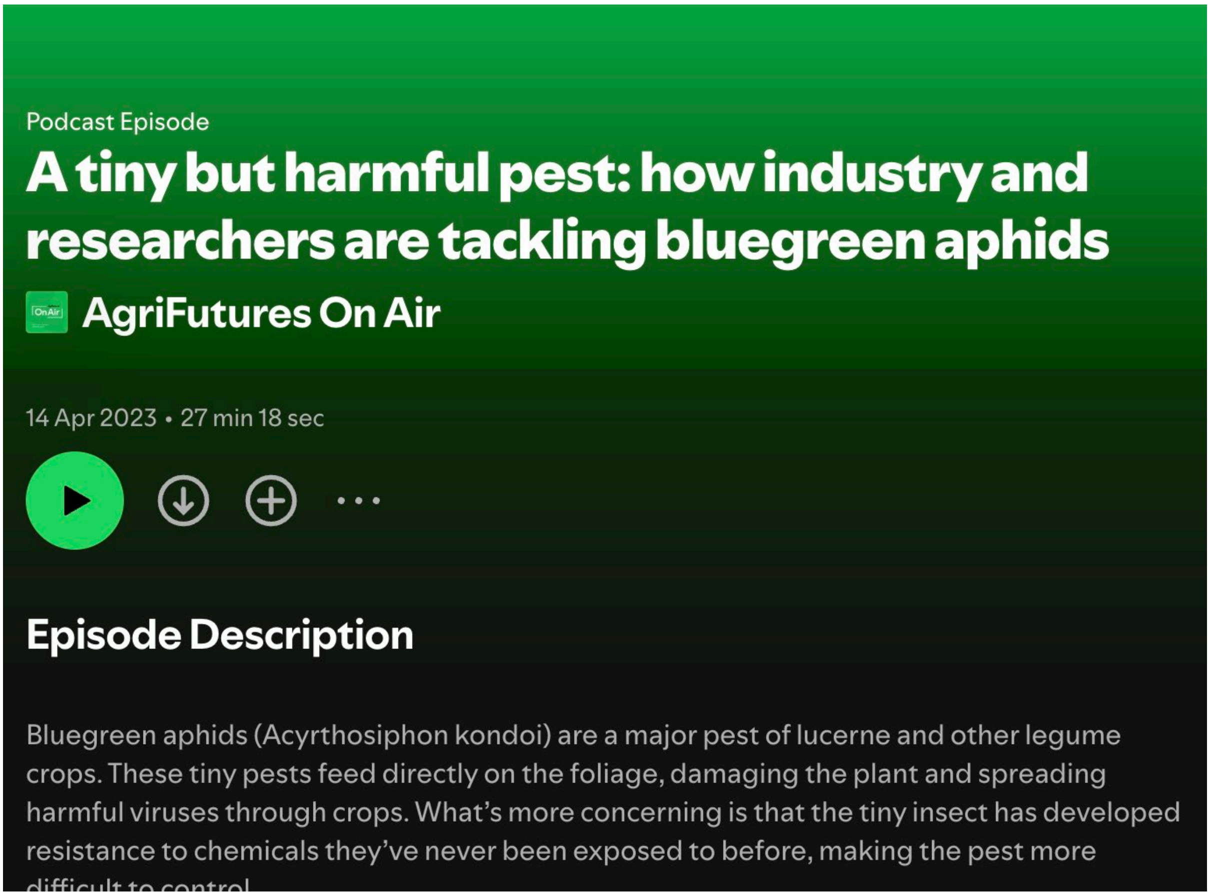
Pasture seed cultivars vary in their tolerance to aphid feeding damage (Humphries et al. 2012). Resistant cultivars can help safeguard crops from aphid damage by producing compounds that lower aphid reproduction, decrease preference and/or enhance damage tolerance (Gao et al. 2007). While guides have previously been published to help growers select cultivars for aphid feeding resistance (e.g. NSW DPI 2013), growers rarely consider a cultivar's tolerance to aphid feeding. The yields of aphid-tolerant cultivars are typically lower than those of other (more aphid-susceptible) cultivars. Still, we lack yield data on how these different cultivars perform in years of high aphid density. Additionally, the potential remains to breed aphid-tolerance traits into high-yield cultivars. While such plant breeding programs can take several years, the outcomes of these programs can have long-term benefits to reduce reliance on insecticides.

F9. Investigate the viability of parasitoid augmentation in Australian pasture seed crops

Growers may enhance biocontrol outcomes by augmenting *A. ervi* populations. Augmentation refers to boosting natural populations of biocontrol agents by releasing commercially bred individuals. Augmentation has proven effective in enhancing biocontrol in some crop systems (Parrella et al. 1992, Collier and Van Steenwyk 2004), but whether this method is effective and economically viable in Australian pasture seed cropping remains unclear. So far, augmentation has shown the most success in high-value and protected area cropping systems (Ridland et al. 2020). As such, the application of augmentation at a broadacre scale poses logistical and economic unknowns that require further assessment. However, given *A. ervi* is already in mass production by Australian biocontrol breeders, there are promising opportunities to explore these unknowns.

Appendices

A selection of screenshots and images for some of the project's extension and communication outputs listed in Table 7.



INSECTICIDE RESISTANCE IN BLUEGREEN APHID SPREADS INTO NEW REGIONS OF VICTORIA AND SOUTH AUSTRALIA

by Lillia Jenkins, Julia Severi, and Evatt Chirgwin, Cesar Australia

As autumn approaches, growers will encounter a greater risk of bluegreen aphid outbreaks in lucerne and other legume crops.

The emergence and spread of insecticide resistance in bluegreen aphids may make controlling these outbreaks more challenging than in previous years.

In this article, we report on new research that shows insecticide-resistant bluegreen aphids have spread further across southern Australia than previously recognised.

First-of-its-kind insecticide resistance

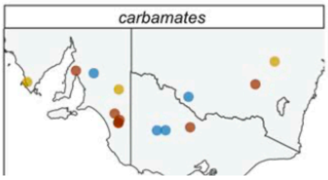
The bluegreen aphid (*Acyrthosiphon kondoi*) is a pest of lucerne, pulses, medics, clovers, and pastures. Outbreaks of this tiny pest

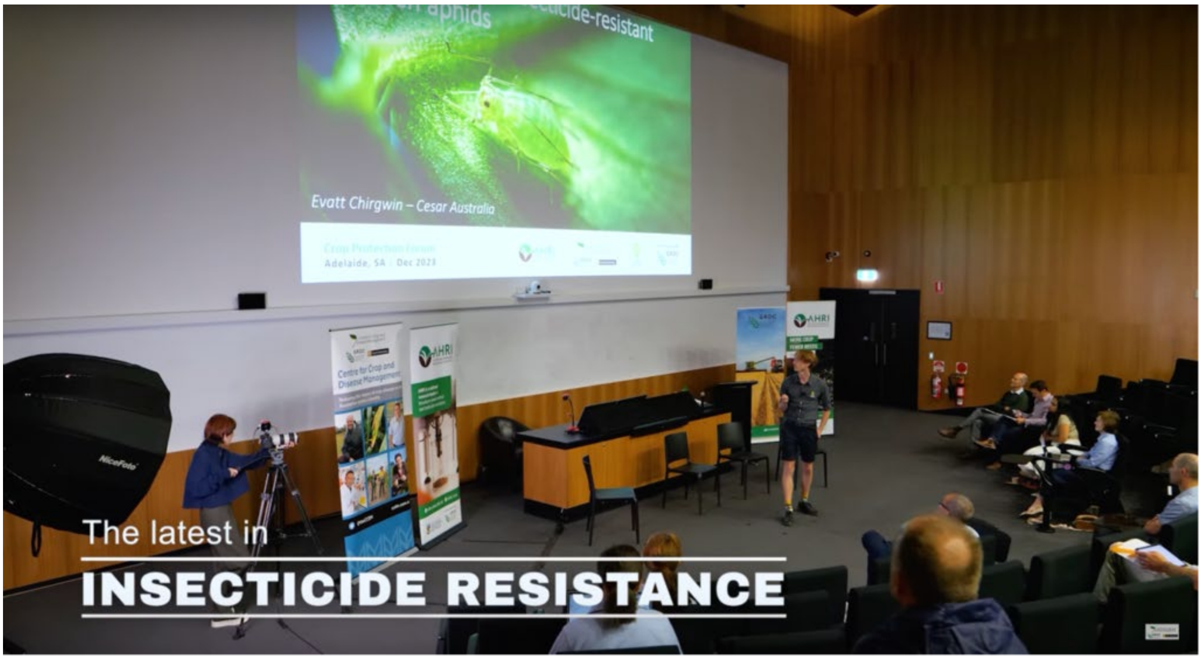
Key unknowns remain about what this first-of-its-kind evolutionary emergence means for management, including the risk of future control failures, where resistant strains have spread, and the long-term efficacy of other insecticides used in bluegreen aphid control.

A new project researching insecticide resistance

Cesar Australia and Lucerne Australia have commenced parallel research projects, with investment from AgriFutures Australia and the GRDC, to help pasture seed and pulse growers manage insecticide resistance in bluegreen aphid. To do so, we are gathering information on these resistant populations, including where they have spread and what crop types they are present in.

Beginning in Spring 2022, lead research scientist Dr. Evatt Chirgwin and his team have been collecting bluegreen aphid populations originating from across southern Australia. Many of these populations were obtained with the help of growers and agronomists – who posted bluegreen aphid samples from their paddocks to Cesar Australia's lab.





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Discovery of insecticide resistance in field-collected populations of the aphid pest, *Acyrthosiphon kondoi* Shinji

Evatt Chirgwin,[a*] Joshua A Thia,[b] Katrina Copping[c] and Paul A Umina[a,b*]

Abstract

BACKGROUND: The bluegreen aphid (*Acyrthosiphon kondoi*) is a worldwide pest of alfalfa, pulses, and other legume crops. An overreliance on insecticides to control *A. kondoi* has potentially placed populations under selection pressure favouring resistant phenotypes, but to date, there have been no documented cases of insecticide resistance. Recently, Australian growers began reporting that conventional insecticides were failing to adequately control *A. kondoi* populations, prompting this laboratory-based investigation into whether these populations have evolved resistance.

Insecticide resistance in bluegreen aphid spreads into new regions of south-eastern Australia

March 7, 2023

Lilia Jenkins

Evatt Chirgwin

As autumn approaches, growers will encounter a greater risk of bluegreen aphid outbreaks in legume crops.

The emergence and spread of insecticide resistance in bluegreen aphid may make controlling these outbreaks more challenging than in previous years.

In this article, we report on new research that shows resistance in bluegreen aphid may have spread further across south-eastern Australia than previously recognised.

First-of-its-kind insecticide resistance

The bluegreen aphid (*Acyrthosiphon kondoi*) is a pest of lucerne, pulses, medics, clovers, and pastures. Outbreaks of this tiny pest are most common in spring and autumn, where they damage plants by feeding on foliage and spreading plant viruses.

New biocontrol research

Biocontrol can be effective at suppressing aphid populations and will help lessen the risk of further insecticide resistance evolving in the future.

Growers and agronomists are increasingly harnessing naturally occurring predators (i.e., natural enemies) of pests as a key component of their pest management strategies. However, controlling bluegreen aphids with natural enemies has some challenges, because besides generalist aphid predators (e.g., ladybugs & lacewings) limited information is available on which naturally occurring species attack bluegreen aphids.

Cesar Australia and Lucerne Australia are investigating one of the most effective and widespread groups of natural enemies of aphids – parasitoid wasps. Parasitoid wasps deposit their eggs inside living aphids, which then hatch and eat their aphid hosts from the inside out: forming aphid mummies. When the wasp is fully developed, it emerges through the aphid shell, and flies off to repeat the cycle.

The bloated golden-bronze appearance of a parasitised aphid: also known as an aphid mummy. Photo by Andrew Weeks, Cesar Australia

Bluegreen aphid samples sought

GROWERS and agronomists are being asked to send samples of bluegreen aphids after research confirmed further spread of insecticide-resistant populations.

The bluegreen aphid is commonly found in lucerne, clover and other legume crops. It feeds directly on foliage, damages the plant and can spread harmful viruses that devastate crops.

Samples should be sent in non-crushable plastic containers and include at least 50 non-disturbed leaf samples on a piece of paper towel.

Use an overnight express post bag and send samples to Bluegreen aphid resistance testing servicE, Evatt Chirgwin, Cesar Australia, Level 1, 95 Albert Street, Brunswick Victoria 3056.

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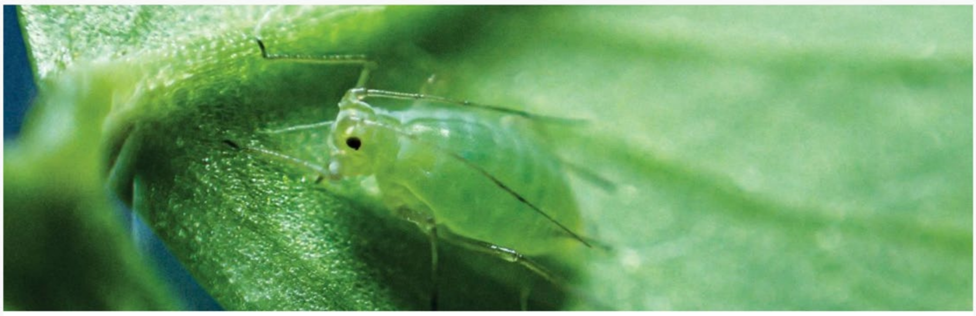
Pressure building to find new control strategies for aphids



There is increasing incidence of insecticide resistance among blue-green aphid.
Photo: Evatt Chirgwin, Cesar Australia

The need for an integrated strategy to control blue-green aphids has been heightened by the discovery of new pesticide-resistant populations in lentil crops in Victoria's Wimmera and Mallee regions.

Recent research led by Dr Evatt Chirgwin (Cesar Australia) found new resistant populations in these important pulse-growing

Key points

- An integrated strategy to control blue-green aphids is needed with increasing incidence of insecticide resistance
- Blue-green aphids (*Acyrthosiphon kondoi*) are common pests of pulses and pastures, damaging plants through

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Understanding and developing a response to bluegreen aphid resistance to chemical controls
Final report

By Evatt Chirgwin, Lilia Jenkins, Karyn Moore, Aston Arthur, Danielle Lannin England and Paul Umina

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