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Assessing the sub-lethal impacts of insecticides on aphid parasitoids through laboratory-based studies

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Abstract

Aphids are a major pest of cropping systems throughout the world. In most cases, crop aphids are controlled with broad-spectrum insecticides; although generally very effective at preventing yield loss, this approach risks non-target damage to beneficial organisms. In the last 20 years, a number of selective insecticides have become available to control aphids while minimising harm to other arthropods. Previous studies have found that two such insecticides, flonicamid and afidopyropen, cause only low-level acute mortality impacts on aphid parasitoids in Australian grain crops. However, little research has examined the sub-lethal effects of these chemicals, which could induce various physiological changes that impact pest control. We hypothesised that both flonicamid and afidopyropen have negative effects that extend beyond the immediate acute mortality previously published. To test this hypothesis, we undertook a series of experiments to determine the effects of flonicamid and afidopyropen, along with the synthetic pyrethroid gamma-cyhalothrin, on aphid parasitism (mummification) rate, emergence rate of the next generation and the next generation sex ratio in three important aphid parasitoids, *Aphidius colemani* (Viereck), *Diaeretiella rapae* (M'Intosh) and *Aphelinus abdominalis* (Dalman). Analogous with previous research, our acute toxicity bioassays showed that all three insecticides had low (<30%) mortality impacts. Although sub-lethal impacts could not be assessed for *D. rapae* due to the low level of aphid parasitism by that species, our findings showed negative impacts on fecundity in surviving *A. abdominalis* and *A. colemani*. Of particular note is the increase in International Organisation for Biological Control ratings to moderate (30%–80% mortality and/or reproductive reduction) when mortality and reduced fecundity effects were combined to determine overall fitness impacts. Gamma-cyhalothrin typically resulted in higher negative impacts on *A. abdominalis* and *A. colemani* (compared with flonicamid and afidopyropen); however, quite surprisingly, these impacts were not rated as highly toxic. Taken together, our results suggest that, even when sub-lethal impacts are considered, flonicamid and afidopyropen are useful tools for farmers targeting aphid populations while minimising the non-target impacts on parasitoids. We recommend semi-field and/or field trials to further assess the impacts of these insecticides on aphid parasitoid populations.

KEYWORDS
Aphelinus abdominalis, *Aphidius colemani*, biological control, *Diaeretiella rapae*, pesticides

INTRODUCTION

Aphids are a major pest of crops throughout much of the world, causing significant economic damage through direct feeding, excretion of honeydew and virus transmission (Dedryver et al. 2010). Typical control methods centre on the application of broad-spectrum insecticides (Frewin et al. 2012; Ricupero et al. 2020), which, while usually effective at controlling pest populations and preventing yield losses, can have a range of negative environmental impacts, including damage to non-target organisms (Siviter & Muth 2020). An example of non-target damage is the impact on beneficial arthropods, including predators and parasitoids that can themselves play an important role in pest control (Holloway et al. 2008). Additionally, overuse of these chemicals can drive the evolution of insecticide resistance amongst pests, such as aphids (Micic et al. 2008; Umina et al. 2019). Thus, there are substantial gains to be made by developing integrated pest management (IPM) strategies for the control of aphids, which utilise a range of chemical and biological pest control methods (Micic et al. 2008; Ricupero et al. 2020; Umina et al. 2019).

To control aphids while preserving biological control agents, a number of selective insecticides have been developed, which putatively have minimal impact on beneficial arthropods, including aphid predators and parasitoids (Ishaaya et al. 2007). Two recent examples are flonicamid and afidopyropen. Flonicamid (Mode of Action Group 29) was first registered for use in Japan in 2006 and is now widely used globally, being registered in 41 countries by 2014. This chemical works by disrupting the aphid nervous system, inhibiting feeding and ultimately leading to death by starvation (Morita et al. 2007). Although from a different mode of action group (Group 9D), afidopyropen also works through neural disruption and prevents feeding by aphids (Horikoshi et al. 2022). In 2018, Australia became the first country to register afidopyropen for use as an insecticide (Australian Pesticides and Veterinary Medicines Authority [APVMA] 2023) and it has since been registered in several other countries with large agricultural sectors including China, India and the United States (Horikoshi et al. 2022).

Within Australia, grain crops (including canola, cereals and pulses) are severely impacted by aphids and their associated viruses (Hoffmann et al. 2008; Valenzuela & Hoffmann 2015). Importantly, however, a wide range of arthropods found in Australian cropping systems attack aphids (Ward et al. 2021b), including at least 10 species of primary parasitoid wasps (Ward et al. 2021a; Waterhouse & Sands 2001). Several species of aphid parasitoids are also commercially produced in Australia for use in augmentative biological control programmes (Holloway et al. 2008). However, despite this abundance of potential biological control agents, IPM programmes are rarely used in Australian grains, with growers primarily relying on insecticide-based treatment programmes (Umina et al. 2019).

Working with three commercially important species of Australian aphid parasitoids—*Aphidius colemani* (Viereck Hymenoptera: Braconidae), *Diaeretiella rapae* (M'Intosh, Hymenoptera: Braconidae) and *Aphelinus abdominalis* (Dalman, Hymenoptera: Aphelinidae)—Overton et al. (2023) elucidated the acute toxicity of 20 insecticides widely used in Australian grain crops. This work included assessments of the impact of flonicamid and afidopyropen, which were shown to have low impact (defined by the International Organisation for Biological Control—IOBC—as below 30% mortality; Sterk et al. 1999) on all three parasitoids, consistent with international field-based studies (e.g., Slusher et al. 2021). This work also reported a surprising finding for the synthetic pyrethroid, gamma-cyhalothrin (Mode of Action Group 3A). Synthetic pyrethroids have generally been found to be non-selective and highly toxic to beneficial arthropods (e.g., Akol et al. 2002; Sugiyama et al. 2011); however, Overton et al. (2023) found gamma-cyhalothrin to have low to moderate toxic impacts on all three species.

The IOBC recommends that, when lethal effects of an insecticide on a beneficial organism are low, further research should be undertaken to determine whether the chemical has sub-lethal impacts on the individuals that survive exposure. Sub-lethal impacts are any impacts experienced by individuals that survive exposure to some form of stress, such as contact with an insecticide, that can limit their beneficial role within the ecosystem. They include, but are not limited to, impacts on reproductive output, fitness, longevity of offspring (Hassan et al. 1985) and changes in the sex ratio of the next generation (Desneux et al. 2007). To date, studies examining the sub-lethal effects of flonicamid and afidopyropen on aphid parasitoids remain scarce. A recent study examined the lethal and sub-lethal (reduced reproduction) impacts of afidopyropen, applied at rates typical for the Australian horticulture industry, to *A. colemani* and found combined impacts that are considered 'moderate' based on IOBC ratings (Hort Innovation 2020), while studies on other parasitoid species have found that flonicamid has low sub-lethal impacts (e.g., Fernández et al. 2015; Karmakar & Shera 2020). Thus, we hypothesised that both flonicamid and afidopyropen have negative effects that extend beyond the immediate acute mortality that was previously assessed for the aphid parasitoids *A. colemani*, *D. rapae* and *A. abdominalis*.

To test this hypothesis, we investigated the sub-lethal effects of flonicamid and afidopyropen on these three parasitoids. Given the surprising findings of Overton et al. (2023) regarding the impacts of gamma-cyhalothrin, we also investigated the sub-lethal effects of this chemical. Our specific aim was to quantify the effects of flonicamid, afidopyropen and gamma-cyhalothrin on the reproductive output and offspring sex ratio of *A. colemani*, *D. rapae* and *A. abdominalis* under controlled laboratory conditions. While this study reflects application rates used in Australian grain crops, the chemicals examined are widely used at the same or similar rates for aphid control globally, and so these results have relevance in other countries and contexts.

MATERIAL AND METHODS

Insects

Aphidius colemani, *D. rapae* and *A. abdominalis* were obtained from the commercial insectary, Biological Services (Loxton, South Australia), and were received as aphid 'mummies' (swollen and discoloured aphid carcases indicative of the presence of an internal parasitoid pupa). Mummies were transported to the laboratory via overnight post and upon receipt were transferred to unlidded 60-mm-diameter petri dishes and placed inside insect-rearing cages (60 × 60 × 120 cm in size; BugDorm, MegaView Science, Taichung, Taiwan). These cages were kept at ∼20°C until adult emergence, at which time parasitoids were provided with damp paper towel as a water source and 100% honey smeared on paper towel as a food source. *Aphidius colemani* and *A. abdominalis* were received in green peach aphid (*Myzus persicae* Sulzer) mummies, while *D. rapae* were received in turnip aphid (*Lipaphis pseudobrassicae* Davis) mummies. To ensure that *D. rapae* individuals used were not naive to *M. persicae* prior to the start of each bioassay, a bok choi (*Brassica rapa* subsp. *chinensis* L.) plant heavily infested with *M. persicae* individuals was introduced to the *D. rapae* insect-rearing cages, positioned so that leaves hung ∼1 cm above each petri dish.

We examined the impact of the target chemicals on each wasp species through their interaction with *M. persicae*. The aphids used in this study originated from a population collected from a canola crop (*Brassica napus* subsp. *napus*) located near Forbes (New South Wales, Australia) that had been cultured on radish (*Raphanus raphanistrum* subsp. *sativus* L.) cotyledons for ∼2 years prior to our bioassays. This population is free of secondary endosymbionts known to impact resistance to parasitism in *M. persicae* (Yang et al. 2023). Approximately 1 month prior to each bioassay, aphids were 'bulked' on bok choi plants before being age matched to suit the parasitoid species (see below).

Acute chemical toxicity bioassays

Parasitoid wasps were exposed to each insecticide following the methods of Overton et al. (2023), which were based on IOBC protocols for residual toxicity assays (Sterk et al. 1999). The three parasitoid species were tested separately, but identical methods were used in each bioassay.

Individual parasitoids of each species were 24–48 h old at the time of insecticide exposure. Only female parasitoids were used in the bioassays; however, female parasitoids were retained in mixed sex populations within the insect-rearing cages for at least 24 h after emergence. Thus, all female parasitoids were assumed to have mated prior to the bioassays, given that mating typically takes place immediately after emergence (Stary 1970).

Insecticide solutions of flonicamid, afidopyropen and gamma-cyhalothrin were applied to 60-mm-diameter plastic petri dish bases using a Potter Tower (Burkard Manufacturing Co. Ltd., Rickmansworth, UK) at a spray deposit rate of 1 mg/cm^2, which is equivalent to a field application of 100 L/ha. Each chemical was tested at a single rate representing the maximum registered field rate (MRFR) for that chemical in Australian grain crops (Table 1). Along with the three insecticide treatments, a negative control was included in all bioassays, which was identical to the insecticide treatments, except that petri dishes were sprayed with distilled water only. For each species and chemical treatment, 10 replicate petri dishes were established.

Following application, petri dishes were left to dry for 30–60 min at room temperature, after which point three female parasitoids were placed in each petri dish (i.e., a total of 30 individual wasps were tested per treatment). Parasitoids were added by placing them on an unsprayed petri dish lid, along with a wick soaked in 10% honey solution, and inverting the sprayed base on top of the lid. Neither the parasitoids nor the wick was placed in direct contact with the sprayed surface. Petri dishes were ventilated to allow for air flow following IOBC recommendations. Dishes were stored within air-tight, transparent 80-L plastic containers, at ∼20°C, with a 14.5:9.5-h light/dark photoperiod and relative humidity maintained at ∼95%. After 72 h, the total number of alive and dead parasitoids in each petri dish was recorded. Although numerous studies assess parasitoid mortality after 48 h, we chose 72 h as the endpoint because our earlier work showed that

TABLE 1 Chemical treatments and application rates used in each bioassay.

Active ingredient	Trade name	Manufacturer	Application rate used (g a.i./ha)
Control (water only)	—	—	—
Flonicamid	Mainman™	ISK Biosciences	50.0
Afidopyropen	Verys™	BASF Australia	5.0
Gamma-cyhalothrin	Trojan™	FMC	4.5

treatment mortality often increases between 48 and 72 h while the control mortality remains relatively stable over this period (Overton et al. 2023).

Mummification, adult emergence and sex-ratio responses

At the conclusion of each acute chemical toxicity bioassay, 20 surviving parasitoids from each chemical (and water) treatment, selected at random, were transferred to aphid arenas, one parasitoid per arena. Each arena consisted of a 35-mm-diameter petri dish with 20 *M. persicae* on a small radish leaf set into 1% agar. Each arena was considered a separate replicate. To ensure that aphid hosts were most suitable to the parasitoids, individuals were age matched to the preferred host age for each wasp species; for *A. colemani* and *A. abdominalis*, the preferred age is 1–3 days old (first to second instar; Perdikis et al. 2004; Suck et al. 2012), and for *D. rapae*, the preferred age is 3–6 days old (third to fourth instar; Ghorbanian et al. 2019).

Three days after parasitoid introductions (3 DAI), aphid mortality was assessed, and all parasitoids were removed from the arenas. Aphid mortality, aphid mummification and emergence of adult parasitoids from mummies were assessed again at 5, 10, 15, 20 and 30 DAI, with living aphids transferred to new radish leaves set into 1% agar at 5, 10 and 15 DAI. Arenas were maintained at ~20°C throughout the experiment.

At 10, 15, 20 and 30 DAI, all dead aphids were removed from arenas and retained in separate petri dishes to observe possible development of mummification while clearly distinguishable mummies were removed from leaves using a paintbrush and kept in separate petri dishes on a piece of filter paper. Any newly emerged aphid nymphs were removed at each assessment to prevent their presence biasing counts of parasitism. Mummification assessments ended at 20 DAI and assessments of emerged adults ended at 30 DAI (these timings were based on the literature and pilot studies that demonstrated that this was more than sufficient time to ensure that no mummies or emerging adults were missed). Emerged adult wasps were examined under a dissecting microscope to determine their sex (as per Ward et al. 2021a), and the sex ratio of this F1 generation was calculated.

Statistical analysis

Response variables

Parasitoid mortality was measured as the proportion of parasitoid wasps that died during the 72-h period of exposure in the acute chemical toxicity bioassays. Mummification rate was measured as the proportion of aphids that became mummies. While each replicate started with the same number of aphids, mortality and escapees resulted in differing numbers being present in each replicate as the experiment progressed. Thus, mummification rate was standardised against the number of aphids surviving at 5 DAI, which was the last assessment day that aphids were examined prior to mummies becoming apparent in any treatment. Adult emergence was measured as the proportion of aphids surviving at 5 DAI in each replicate from which adult parasitoids emerged. Sex ratio was measured as the number of female wasps to emerge in each treatment as a proportion of total number of wasps emerged.

In addition, we calculated two fitness cost metrics to capture the combined effect of lethal (i.e., reduced survival due to acute toxicity) and sub-lethal effects (i.e., reduced fecundity due to reduced mummification and emergence rates). Similar metrics are commonly used to illustrate the population-level effects insecticides could be expected to produce when a proportion of a population suffers acute mortality and the surviving proportion suffers sub-lethal effects such as reduced fecundity (e.g., Hort Innovation 2020). These metrics were conceptualised under the multiplicative fitness model and defined as

$$\text{Reduction in parasitism capacity (RPC)} = \begin{array}{l} 1-(\text{survival rate relative to water control} \\ \times\ \text{mummification rate relative to water control}). \end{array}$$

$$\text{Reduction in reproductive capacity (RRC)} = \begin{array}{l} 1-(\text{survival rate relative to water control} \\ \times\ \text{emergence rate relative to water control}). \end{array}$$

Here is an example to illustrate the calculation of the fitness costs metrics using acute mortality and mummification rate data for *A. abdominalis* after exposure to afidopyropen. The mean acute mortality of *A. abdominalis* was 0.101 for afidopyropen and 0.001 for the control treatment. Thus,

$$\text{survival rate is } (1-0.101)/(1-0.001)=0.90.$$

The mean mummification rate of *A. abdominalis* was 0.193 for afidopyropen and 0.494 for the control treatment. Thus,

$$\text{mummification rate is } 0.193/0.494=0.39.$$

So, the RPC is 1 − (0.90 × 0.39) = 0.65. This represents a 65% fitness reduction in *A. abdominalis* relative to control population.

Modelling approach

We used logistic regression models to assess acute parasitoid mortality, and aphid mummification and parasitoid emergence rates. For each parasitoid species, we specified the mortality model as

$$\mathrm{Mortality}_i \sim \mathrm{Binomial}\,(N_i, p_i)$$

where p_i is the probability that the tested N_i wasps in each replicate petri dish *i* will die during the 72-h exposure period.

We specified the mummification models as

$$\mathrm{Mummification}_i \sim \mathrm{Binomial}\,(N_i, p_i)$$

where p_i is the probability that the examined N_i aphids in each replicate petri dish *i* will develop into mummies.

We specified the emergence models as

$$\mathrm{Emergence}_i \sim \mathrm{Binomial}\,(N_i, p_i)$$

where p_i is the probability that (a) one or more adult parasitoids will emerge from the observed N_i mummies in each replicate petri dish *i* (adult emergence model) and (b) one or more of the observed N_i emerged parasitoids will be a female (female emergence model).

The linear predictors were specified on the logit-probability scale as

$$\text{Mortality rate models} : \mathrm{logit}(p_i) = \mathrm{alpha}[[t_i]].$$

$$\text{Mummification rate models} : \mathrm{logit}(p_i) = \mathrm{beta}[[t_i]].$$

$$\text{Emergence rate models} : \mathrm{logit}(p_i) = \mathrm{gamma}[[t_i]].$$

$$\text{Female emergence rate models} : \mathrm{logit}(p_i) = \mathrm{delta}[[t_i]].$$

where t_i is the integer indexing the four treatments (1 = control, 2 = afidopyropen, 3 = flonicamid and 4 = gamma-cyhalothrin); alpha, beta, gamma and delta are the treatment-level fixed effects, which were assigned to non-informative priors Normal (mean = 0, SD = 32); and logit is a link function defined as $\mathrm{logit}(x) = \ln(x/1 - x)$.

RPC and RRC were estimated as

$$\mathrm{RPC} = 1 - (\mathrm{survival} \times \mathrm{ilogit}(\mathrm{beta}[[t_{2:3}]]/\mathrm{beta}[[t_1]]))$$

and

$$\mathrm{RRC} = 1 - (\mathrm{survival} \times \mathrm{ilogit}(\mathrm{gamma}[[t_{2:3}]]/\mathrm{gamma}[[t_1]]))$$

where $\mathrm{survival} = (1 - \mathrm{ilogit}(\mathrm{alpha}[[t_{2:3}]]/\mathrm{alpha}[[t_1]]))$ and the inverse function ilogit is defined as $\mathrm{ilogit}(x) = \exp(x)/(1 + \exp(x))$ (Kéry & Royle 2016).

As alpha, beta and gamma were estimated within a Bayesian inference framework, the RPC and RRC estimates were derived with their full associated uncertainties, which allowed us to statistically compare them across species and treatments.

Bayesian inference implementation

We estimated all model parameters under Bayesian inference, using Markov chain Monte Carlo (MCMC) simulations to draw samples from the parameters' posterior distributions. We implemented models in JAGS (Plummer 2003), as accessed through the R package jagsUI (Kellner 2016). We used three chains of 5000 iterations, discarding the first 500 in each chain as burn-in. We visually inspected the MCMC chains and the values of the Gelman–Rubin statistic to verify acceptable convergence levels of $\hat{r} < 1.1$ (Gelman & Hill 2007).

RESULTS

Acute chemical toxicity

For *A. colemani*, *D. rapae* and *A. abdominalis*, mortality after 72 h of exposure to each of the three chemical treatments was in the range that would be considered 'low' based on IOBC ratings (i.e., below 30%—Sterk et al. 1999) (Figure 1). Within this range, the level of mortality for all three species after exposure to gamma-cyhalothrin was statistically higher than the control treatment. Mortality following exposure to afidopyropen was statistically higher than the control treatment for *A. abdominalis* but not for *A. colemani* or *D. rapae*, while mortality following exposure to flonicamid was not statistically different to the control treatment for any of the three species.

Aphid mummification rate

The aphid mummification rate for *A. abdominalis* was statistically lower following exposure to afidopyropen and gamma-cyhalothrin compared with the control treatment. The mean mummification rates for *A. abdominalis* were 49%, 19% and 17% in the water control, gamma-cyhalothrin and afidopyropen treatments, respectively (Figure 2). The mean mummification rate in the flonicamid treatment for this species was 36%, which was not statistically different to the control treatment (Figure 2). The mean mummification rate for *A. colemani* was 18% in the control treatment, much lower than observed for *A. abdominalis*. For *A. colemani*, the mean mummification rates for afidopyropen, flonicamid and gamma-cyhalothrin were 15%, 11% and 14%, respectively. None of these rates were statistically different to the control treatment (Figure 2). The mean mummification rate for

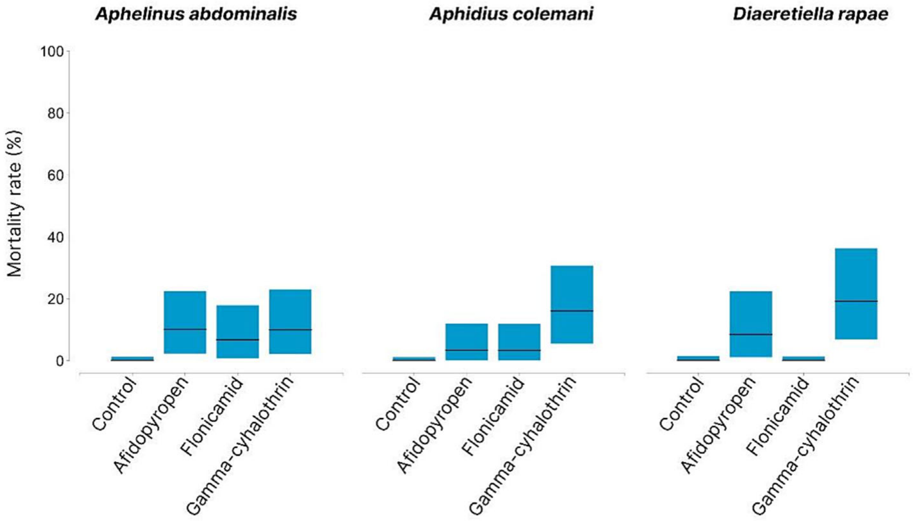

FIGURE 1 Mortality for each parasitoid species following exposure to each treatment after 72 h. The horizontal black lines represent the mean mortality across all replicates, and the blue boxes represent the 95% credible intervals.

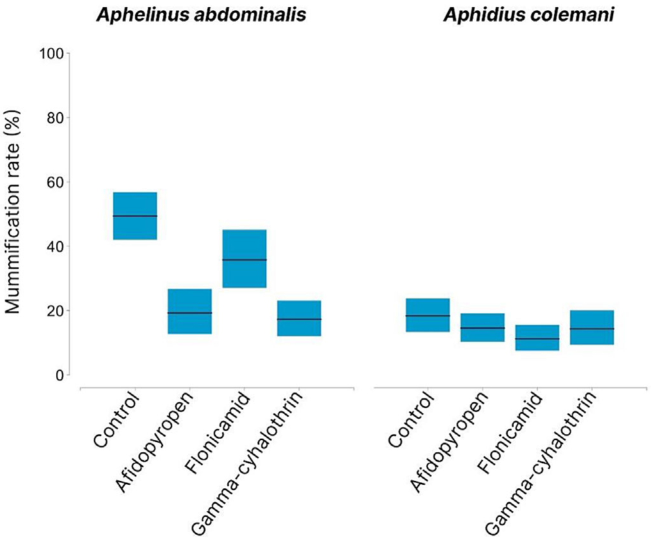

FIGURE 2 Mummification rate of *Aphidius colemani* and *Aphelinus abdominalis* per aphid alive at 5 days after parasitoid introductions. The horizontal black lines represent the mean aphid mummification rate across all replicates, and the blue boxes represent the 95% credible intervals.

D. rapae was only 3% in the control treatment, which precluded any meaningful statistical analysis of mummification rates and subsequent adult emergence data. Thus, *D. rapae* was excluded from the analysis of aphid mummification, adult emergence and sex-ratio responses.

Reduction in parasitism capacity

The RPC measure (which considers both acute mortality and mummification rate) for *A. abdominalis* was 65%, 32% and 68% in the afidopyropen, flonicamid and gamma-cyhalothrin treatments, respectively (Figure 3). As with aphid mummification rates, there was a significant difference in RPC measurements between the flonicamid and gamma-cyhalothrin treatments. For *A. colemani*, the RPC was 26%, 39% and 38% in the afidopyropen, flonicamid and gamma-cyhalothrin treatments, respectively (Figure 3). None of these measures were statistically different from one another.

F1 parasitoid emergence

There was little difference in the emergence rates of F1 parasitoids between treatments for *A. abdominalis*, with mean emergence rates of 19%, 7%, 17% and 9% in the water control, afidopyropen, flonicamid and gamma-cyhalothrin treatments, respectively. In no cases were these differences statistically significant (Figure 4). In the case of *A. colemani*, the emergence rate in the control treatment was 9%, while the emergence rates were 2%, 2% and 3% in the afidopyropen, flonicamid and gamma-cyhalothrin treatments, respectively. The emergence rates following exposure to afidopyropen and flonicamid were statistically lower than the control treatment (Figure 4). Raw emergence figures are included in Table S1.

Reduction in reproductive capacity

The RRC measure for *A. abdominalis* was 67%, 27% and 56% in the afidopyropen, flonicamid and gamma-cyhalothrin treatments, respectively (Figure 5). None of these measures were statistically significant from one another. For *A. colemani*, the RRC was ~75% for each of the afidopyropen, flonicamid and gamma-cyhalothrin treatments (Figure 5).

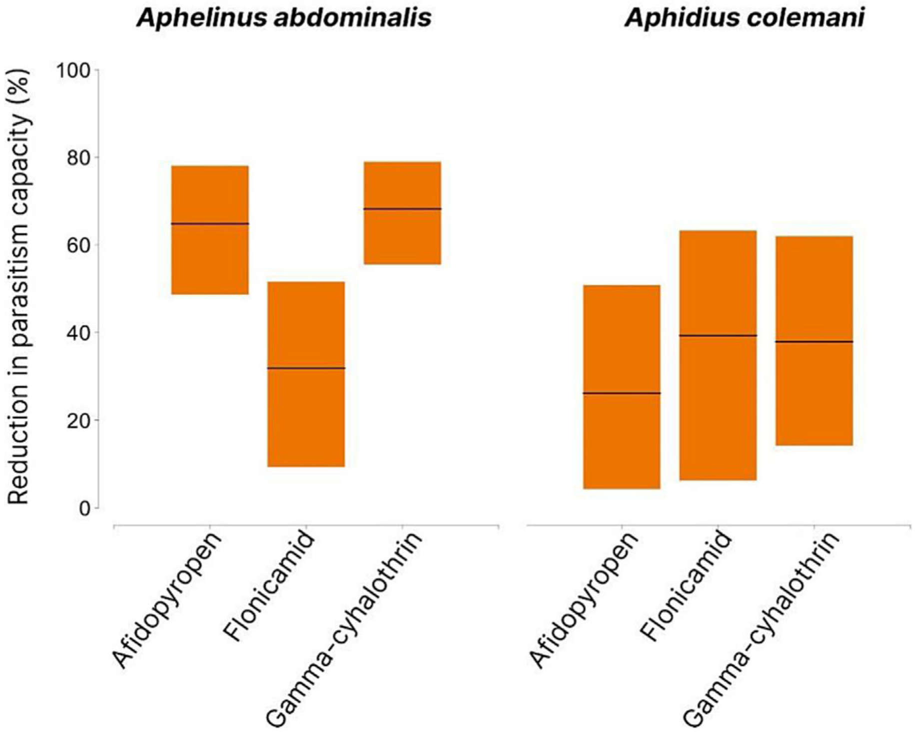

FIGURE 3 Reduction in parasitism capacity estimated for *Aphelinus abdominalis* and *Aphidius colemani* following insecticide exposure. The horizontal black lines represent the mean reduced parasitism capacity across all replicates, and the orange boxes represent the 95% credible intervals.

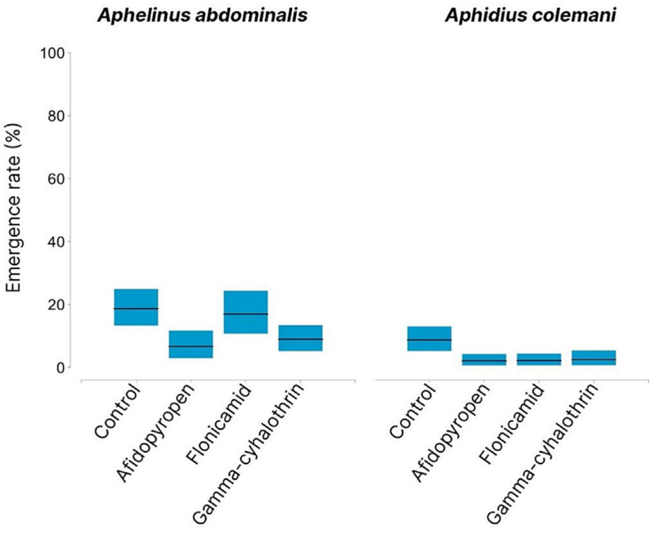

FIGURE 4 Emergence rates of F1 *Aphelinus abdominalis* and *Aphidius colemani* adults following insecticide exposure. The horizontal black lines represent the mean emergence rates across all replicates, and the blue boxes represent the 95% credible intervals.

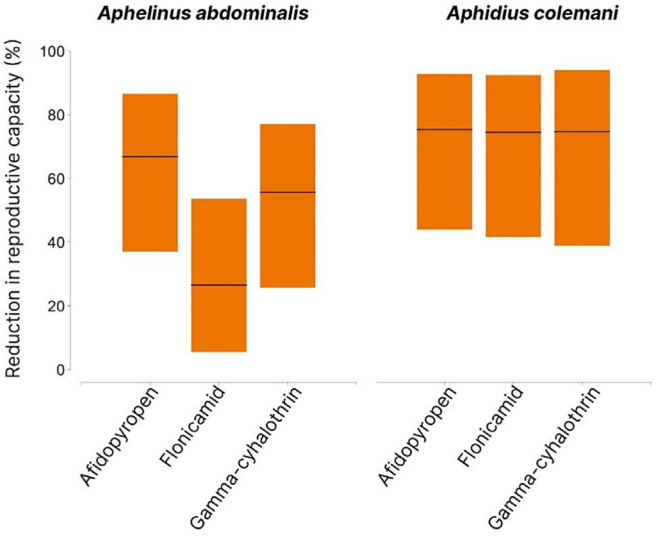

FIGURE 5 Reduction in reproductive capacity estimated for *Aphelinus abdominalis* and *Aphidius colemani* following insecticide exposure. The horizontal black lines represent the mean reduced reproductive capacity across all replicates, and the orange boxes represent the 95% credible intervals.

Sex ratio of F1 parasitoids

The sex ratios of F1 *A. abdominalis* adults that emerged from females exposed to the three insecticides were not statistically different to each other or to the control treatment (Figure 6). For *A. colemani*, the sex ratios of the F1 adults were 50% and 38% in the afidopyropen and control treatments, respectively, but very low in the flonicamid and gamma-cyhalothrin treatments (both ~1%) (Figure 6). The sex ratios in the flonicamid and gamma-

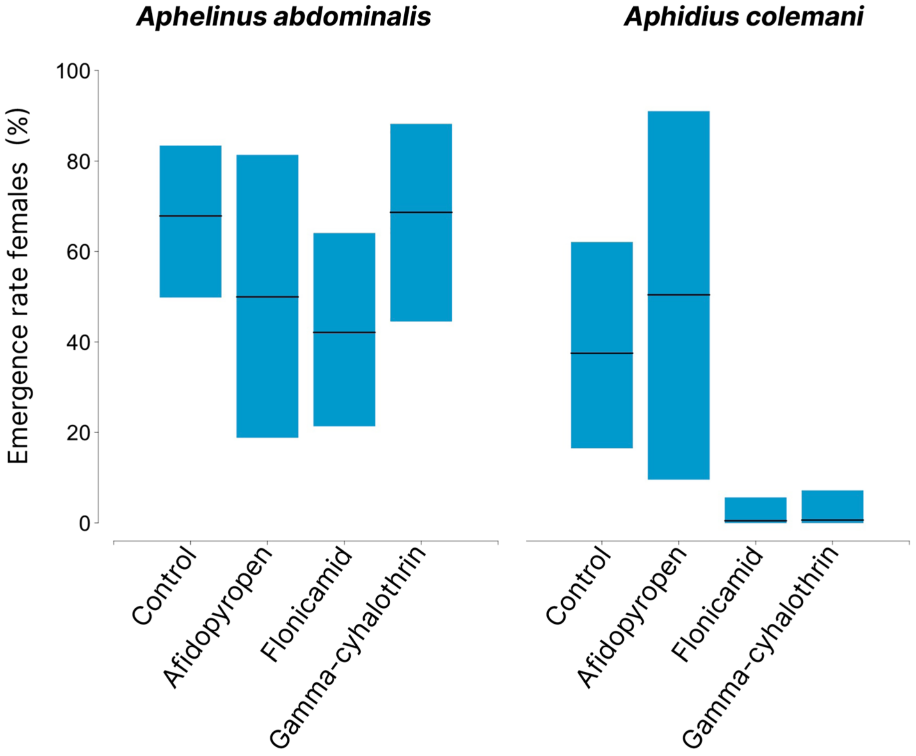

FIGURE 6 The proportion of female F1 *Aphelinus abdominalis* and *Aphidius colemani* adults that emerged from females exposed to insecticides. The horizontal black lines represent the mean emergence rate of females across all replicates, and the blue boxes represent the 95% credible intervals.

cyhalothrin treatments were significantly lower than the water control, although it is important to note that these differences are based on low overall numbers of emerging wasps (see Table S1).

DISCUSSION

Our previous trials examining the acute lethal impacts of various insecticides on *A. abdominalis*, *D. rapae* and *A. colemani* found afidopyropen and flonicamid to be 'low' impact (based on the IOBC rating scale, wherein low is <30% mortality) when applied at the MRFR for Australian grains (Overton et al. 2023), a finding that was replicated here. However, our study provides evidence that, under laboratory conditions, both chemicals have impacts that can extend into the 'moderate' range (defined by the IOBC as 30%–80% mortality and/or reproductive reduction—Sterk et al. 1999) for *A. abdominalis* and *A. colemani* when the combined impacts of acute mortality and reduced reproductive output are considered. We found gamma-cyhalothrin to have a low acute lethal impact on *A. abdominalis*, *D. rapae* and *A. colemani*; however, for all three species, the acute mortality from gamma-cyhalothrin after 72 h was statistically higher than the control treatment. Furthermore, we found a reduction in various measures of reproductive output in both *A. abdominalis* and *A. colemani* following exposure to gamma-cyhalothrin.

Afidopyropen and flonicamid were associated with reduced mummification and adult emergence rates in *A. abdominalis* and *A. colemani*, although these reductions were not always statistically different to the control treatment. Where reductions in parasitism and adult emergence were statistically different from the control, mean values did not exceed the range that would be considered 'moderate' (30%–80%) based on IOBC ratings. This is congruent with a recent study that found that the combined lethal and sub-lethal impacts of afidopyropen on *A. colemani* were in the moderate range (Hort Innovation 2020). The sex ratios of F1 *A. abdominalis* adults were not impacted following exposure to afidopyropen or flonicamid, and the same was true for *A. colemani* following exposure to afidopyropen. However, our results suggest that flonicamid may reduce the proportion of female offspring produced by *A. colemani*. Although caution should be given to this finding given the small number of F1 *A. colemani* adults that emerged in this treatment, this finding highlights the need for further studies to closely examine the sub-lethal impacts of flonicamid.

Although sub-lethal effects were evident in our study, which shifted the overall impact of flonicamid and afidopyropen from the low to moderate level, these findings provide further evidence that both chemicals have a considerably lower impact on aphid parasitoids than many insecticides currently used in Australian grain crops (cf. Overton et al. 2023). This is potentially due to the fact that, despite belonging to different mode of action groups (Insecticide Resistance Action Committee [IRAC] 2020), flonicamid and afidopyropen both inhibit stylet feeding without causing more general knockdown effects (Horikoshi et al. 2022; Morita et al. 2007), thus leaving non-stylet feeding insects, such as parasitoids, relatively unaffected. This is contrary to many broad-spectrum insecticides, which instead act on physiological systems that are important to most animals, such as pyrethroids that stimulate the nervous system more generally, causing hyper-excitation (Ray & Burr 2014).

Our results examining the impacts of gamma-cyhalothrin are analogous with the findings of Overton et al. (2023), who found this chemical to have low-moderate acute lethal mortality on the three species of aphid parasitoids examined here. Those results were unexpected as synthetic pyrethroids (Group 3A insecticides) are typically considered to be non-selective and highly toxic to beneficial arthropods (Akol et al. 2002; Sugiyama et al. 2011). It was thus anticipated that the impact of gamma-cyhalothrin may be higher when sub-lethal effects were considered in our study. Somewhat surprisingly, the combined impacts of acute mortality and reduced reproductive output did not extend the rating of gamma-cyhalothrin on either *A. abdominalis* or *A. colemani* beyond the moderate range (30%–80%). However, as with flonicamid, there does appear to be a significant reduction in the proportion of female offspring produced by *A. colemani* following exposure to gamma-cyhalothrin. It is worth noting that the relatively moderate impact of gamma-cyhalothrin on *A. abdominalis*, *D. rapae* and *A. colemani* as shown in our study is unlikely to be representative of all Group 3A insecticides, as shown previously for these species (Overton et al. 2023) and for lepidopteran parasitoids (Brunner et al. 2001). Other studies involving gamma-cyhalothrin have shown variable results. For example, Beloti et al. (2015) exposed adult *Tamarixia radiata* (Waterston), a psyllid parasitoid, to a low rate of gamma-cyhalothrin and found low (24%) acute lethal mortality and a moderate (75%) reduction in fecundity. Conversely, Carmo et al. (2010) exposed adult *Telenomus remus* (Nixon), a parasitoid of lepidopteran eggs, to a low dose of gamma-cyhalothrin and observed 100% mortality. The variability in impacts of gamma-cyhalothrin is further highlighted by our assessments of acute lethal mortality involving other beneficial arthropods (Knapp et al. 2023). Using the same IOBC testing protocols, we found that gamma-cyhalothrin caused 100% mortality to the parasitoids *Diadegma semiclausum* (Hellen) and *Trichogramma pretiosum* (Riley), along with the lacewing *Mallada signatus* (Schnieder) and predatory bug *Orius tantillus* (Motschulsky). However, rove beetles (*Dalotia* sp.), hoverflies (*Melangyna* sp.) and wolf spiders (*Venatrix* spp.) have been shown to exhibit low mortality following exposure to this chemical (Knapp et al. 2023).

It was somewhat surprising that aphid mummification rates were relatively low in the control treatments for all parasitoid species in our study (especially for *D. rapae*); however, these results are congruous with many previous studies. For example, mummification rates for *A. colemani* on *M. persicae* under laboratory conditions have been shown to be highly variable, ranging from 3% to 80% (Emami et al. 2013; Jaber & Araj 2018; Martinou & Wright 2007; Perdikis et al. 2004). We are not aware of any studies that report mummification rates for *A. abdominalis* on *M. persicae*, although Shrestha et al. (2015) reported 56% mummification for this species on the currant lettuce aphid (*Nasonovia ribisnigri* [Mosley]), a rate that is similar to the 49% observed in our study. Despite implementing measures to reduce the impact of host switching of *D. rapae* from *L. pseudobrassicae* to *M. persicae*, parasitism and subsequent adult emergence in this species remained very low in our study. This is consistent with the findings of Navasse et al. (2018), who observed that mummification rates for *D. rapae* on *M. persicae* were up to 85% lower for parasitoid individuals reared on hetero-specific hosts compared with con-specific hosts. Our findings add support to the hypothesis advanced by both Navasse et al. (2018) and Derocles et al. (2016) that *D. rapae* populations can form strains adapted to specific hosts that may have reduced reproductive output when presented with other host species.

In conclusion, our findings demonstrate that the two selective chemicals, flonicamid and afidopyropen, have negative impacts on the aphid parasitoids *A. abdominalis*, *D. rapae* and *A. colemani* and show that these effects extend to sub-lethal impacts in both *A. abdominalis* and *A. colemani*. Of particular note is the considerable increase in IOBC impact rating when we consider the two fitness cost metrics (RPC and RRC), which enabled us to estimate the combined effect of lethal and sub-lethal chemical effects. Nonetheless, our assays combining acute mortality and reproductive output measures suggest that both flonicamid and afidopyropen have far lower negative impacts than many conventional insecticides used to control pest aphids globally (Garantonakis et al. 2016; Overton et al. 2023; Suma et al. 2009). As a next step, we recommend additional semi-field and/or field trials to thoroughly examine the impacts of these insecticides of aphid parasitoids. As discussed in Jansen et al. (2011), the artificial environment created in laboratory studies is forced and unnatural. Furthermore, the toxicities observed in our study are likely to vary if conducted under different environmental conditions (e.g., temperature and humidity; Abbes et al. 2015; Holmstrup et al. 2010).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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