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Biological and Microbial Control

The effects of insecticide seed treatments on the parasitism and predation of *Myzus persicae* (Homoptera: Aphididae) in canola

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The green peach aphid, *Myzus persicae* (Sulzer) (Homoptera: Aphididae), is a major pest of brassica plants, with the ability to transmit > 100 viruses. Although the adoption of Integrated Pest Management is increasing, chemical treatment remains the predominant method used to control *M. persicae* globally. Insecticide seed treatments, typically with neonicotinoid active ingredients, have become commonplace in canola crops, and are viewed as a "softer" alternative to foliar sprays but may nevertheless impact natural enemies of *M. persicae*. In this study, the effects of canola seed treatments, containing imidacloprid, thiamethoxam, and a mixture of thiamethoxam + lambda-cyhalothrin, were investigated on the parasitoid wasp, *Aphidius colemani* Viereck (Hymenoptera: Braconidae) and the green lacewing, *Mallada signatus* (Schneider) (Neuroptera: Chrysopidae), both important natural enemies of *M. persicae*. Laboratory trials were undertaken using whole plants, with lethal and sublethal effects assessed by measuring several traits. Compared with untreated plants, more aphid mummies were produced and more *A. colemani* were reared on plants treated with thiamethoxam + lambda-cyhalothrin and more aphid mummies were produced on imidacloprid plants. Imidacloprid reduced the time *A. colemani* spent searching for *M. persicae* and thiamethoxam reduced its cleaning time. However, after *A. colemani* were removed from treated plants, there were no such effects observed, suggesting these impacts were relatively short-lived. We found no significant effects of seed treatments on *M. signatus*. These results point to the complexity of ecotoxicology studies involving multiple trophic levels and indicate that seed treatments may have variable impacts on key fitness traits of natural enemies.

Key words: aphid, pest, canola, neonicotinoid, beneficial

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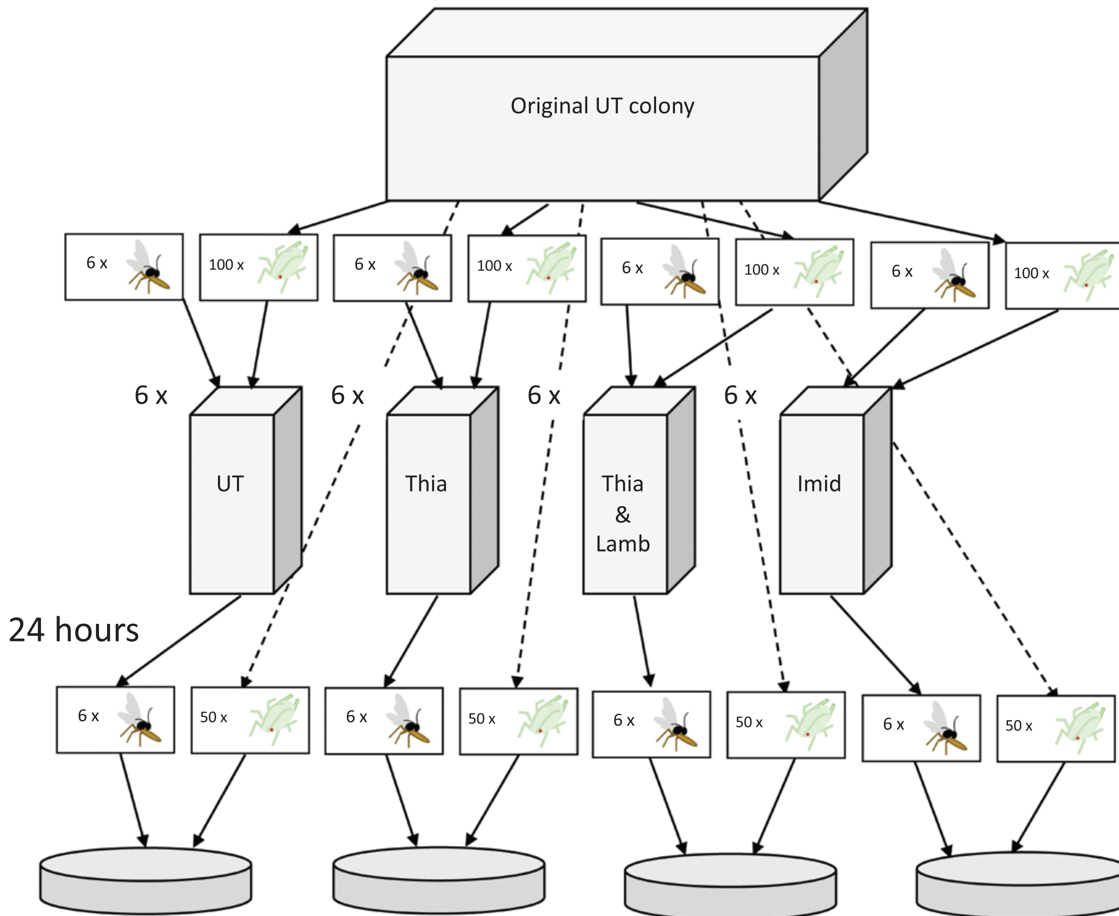
Graphical Abstract



Introduction

The potential negative effects of neonicotinoid insecticides on natural enemies have, for the last decade, been a topic of debate. The leading delivery method of neonicotinoid insecticides in arable agriculture is through seed treatments (Frank and Tooker 2020, Matsuda et al. 2020), which involves the application of an insecticide to the seed, after which the active ingredient moves through the plant systemically. Seed treatments are routinely used for major crops such as maize, canola, soybean, wheat, and cotton (Calvo-Agudo et al. 2021). Rapidly becoming the most widespread of all insecticide groups used in seed treatments (Miao et al. 2014, Douglas and Tooker 2015, Huang et al. 2015, Tooker et al. 2017, Mourtzinis et al. 2019), neonicotinoids were first introduced into the agrichemical market in 1994 with the registration of imidacloprid (Jeschke et al. 2011). Following the success of imidacloprid, other neonicotinoid active ingredients were registered, including acetamiprid, clothianidin, dinotefuran, nitenpyram, thiacloprid, and thiamethoxam (Maienfisch et al. 1999, Maienfisch, Angst, et al. 2001, Maienfisch, Huerlimann, et al. 2001, Morrissey et al. 2015). Neonicotinoids are typically applied to plant seeds as stand-alone treatments, however, there are cases when multiple active ingredients are combined, often involving a mixture of a neonicotinoid and a synthetic pyrethroid. Although both of these chemical groups can cause paralysis and insect death, pyrethroids are sodium channel modulators and are known to possess repellent action toward insects and other arthropods, while neonicotinoids are nicotinic acetylcholine receptor allosteric modulators that act on the nervous system (Burden 1975, He et al. 2008, IRAC 2015, Fernandes et al. 2016).

Neonicotinoid-coated seeds are applied to protect agricultural crops against a broad spectrum of insect (and mite) pests at the start of the cropping cycle with potential economic, as well as environmental benefits (Jeschke et al. 2011, Matsuda et al. 2020). This is generally considered a softer option for chemical control compared with spray applications that cover whole soil or plant surfaces, potentially exposing non-target organisms to the insecticide (Hazra and Patanjali 2016, Sekulic and Rempel 2016). Despite resulting in a more directed delivery, seed treatments may nevertheless have negative effects on natural enemies of pests, which in turn can lead to secondary pest outbreaks (Roubos et al. 2014, Tooker et al. 2017). However, it is not straightforward to predict, or accurately measure, the side effects of insecticides due to many interacting variables (Jepson et al. 1990). This becomes particularly challenging when considering insecticides applied directly to crop seeds, with the efficacy of chemically treated seeds on multiple trophic levels often debated (Walters 2013, Douglas and Tooker 2016, Atwood et al. 2018).

One of the main difficulties in measuring any negative effects of neonicotinoids and other insecticides is the wide range of exposure routes for natural enemies, e.g., predating on prey that have fed on a treated plant or on a product of tainted prey (such as honeydew), through absorption in the soil or local water sources, and/or feeding on extrafloral nectar (Morrissey et al. 2015, Moscardini et al. 2015, Saeed et al. 2016, Eng et al. 2017, Jiang et al. 2018, 2019, Calvo-Agudo et al. 2022). This may be the reason why studies have shown both negative effects of neonicotinoids (Moser and Obrycki 2009, Prabhaker et al. 2011, Seagraves and Lundgren 2012, Gontijo et al.

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2014, Moscardini et al. 2014, 2015, Ohta and Takeda 2015, D'Ávila et al. 2018) as well as very little impact of neonicotinoids on natural enemies (Epperlein and Schmidt 2001, Krauter et al. 2001). Ideally, insecticides should be selective within a specific context (i.e., highly toxic to pests but not to other organisms [Roubos et al. 2014]).

Here we consider the context of canola (*Brassica napus* L.) seed treatments as applied in Australian arable agriculture. As in many countries, Australian canola is almost exclusively sown as insecticide-treated seed with a view to reducing pest threats during the early crop establishment period. The generalist aphid, the green peach aphid, *Myzus persicae* (Sulzer) (Homoptera: Aphididae), is an important pest of canola and other brassica crops in temperate regions of the world (Cole 1997) and is 1 of the 3 major aphid pests infesting canola in Australia (Gu et al. 2007, Ward, Umina, Macfadyen, et al. 2021, Ward, Umina, Polaszek, et al. 2021). *Myzus persicae* often inhabits emerging canola seedlings, which are particularly vulnerable to damage (Moens and Glen 2002). Due to their ability to transmit a number of plant viruses such as turnip yellows virus, *M. persicae* can cause yield losses of up to 50% (Berlandier 2004). Since the early 1950s, insecticide use has been the main method for suppressing *M. persicae* populations (DeBach 1974, Gullan and Cranston 1994, Hardin et al. 1995), which has contributed to this species evolving resistance to more than 80 insecticides, including neonicotinoids and other chemical groups (Bass et al. 2014, Umina et al. 2014, 2022, Mota-Sanchez and Wise 2022, Pym et al. 2022). Therefore, non-chemical biological control for *M. persicae* is considered increasingly important globally.

There are hundreds of natural enemies recorded attacking *M. persicae,* including (mostly generalist) predators, such as ladybird beetles, lacewings and hoverflies, parasitoid wasps (henceforth "parasitoid"), and entomopathogenic fungi. Van Emden et al. (1969) provide an extensive list of these beneficial organisms on a global scale, while Waterhouse and Sands (2001) list 10 species of predator, 6 parasitoids, 8 hyperparasitoids, and 6 fungi, as natural enemies of *M. persicae* in Australia. An important parasitoid species is *Aphidius colemani* Viereck (Hymenoptera: Braconidae), a pan-tropical species, widely distributed in Africa, Asia, Australia, South America, and southern Europe (Starý 1975). This species is commercially available for the biological control of aphids, including *M. persicae* (Grasswitz 1998, Jones et al. 2003). Among the predators, chrysopid species (green lacewings) are widespread and deemed very important (Pappas et al. 2011); their use as biological control agents of aphids has been documented for over 250 years (Senior and McEwen 2001). Chrysopid larvae are voracious aphid predators, and while some adults are also predaceous, others feed on nectar, pollen, and/or aphid honeydew (Pappas et al. 2011). *Mallada signatus* (Schneider) (Neuroptera: Chrysopidae) is a common species native to Australia and New Zealand (Smithers 1988), which is produced commercially to control numerous insect pests, including aphids (Simmons and Gurr 2004).

In this study, we aimed to (i) understand the direct (lethal) effects and indirect (sublethal) effects of insecticide seed treatments on *A. colemani* and *M. signatus* when exposed to *M. persicae* that had fed on insecticide-treated canola plants, and (ii) draw comparisons between the impacts of several canola seed treatments commercially relevant in Australian canola. The goal of this study was to better characterize the effects of seed treatments, thus enabling more informed recommendations to canola growers.

Materials and Methods

Trial Design

We assessed the effects of seed treatments on *A. colemani* across 2 laboratory trials (trials 1a and 1b). The traits measured in these trials

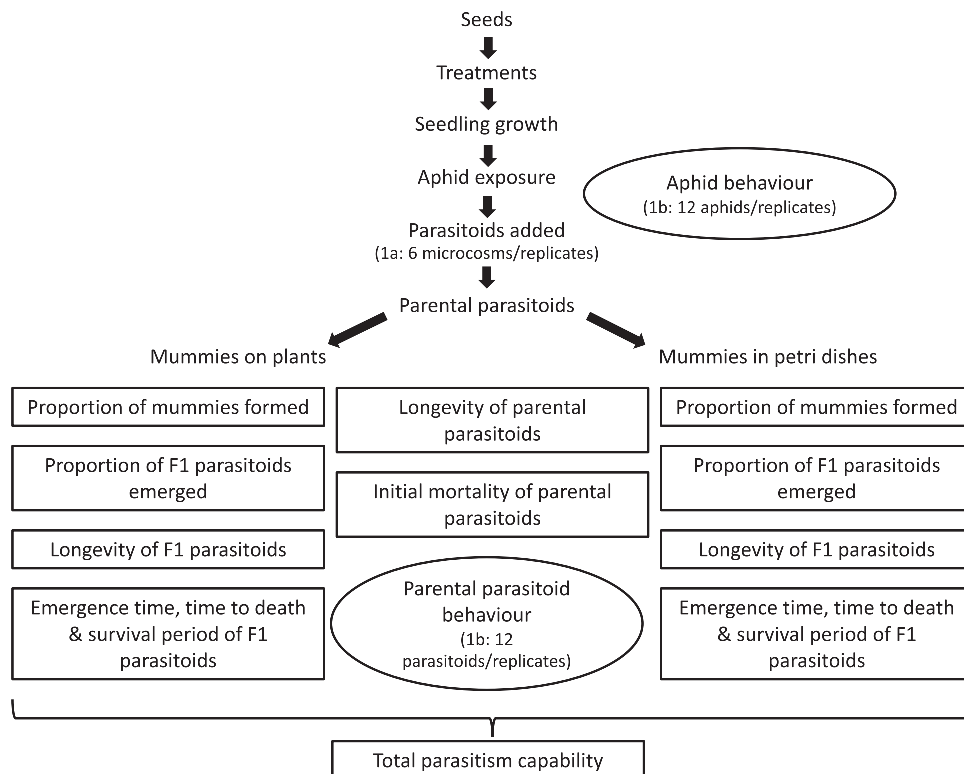

Fig. 1. The traits measured in the *Aphidius colemani* experiment (trial 1), with data collected for trial 1a boxed below each experimental pathway, and data collected for trial 1b circled. Replicates for both trials are noted.

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are shown in Fig. 1 and a schematic outlining the methodology is depicted in Supplementary Fig. S1 for trial 1a and in Supplementary Figs. S2 and S3 for trial 1b. In trial 1a, we measured initial mortality, longevity, and overall population fitness of parental parasitoids. After seed treatment exposure, we measured the number of aphid mummies formed, the number of F1 parasitoids that emerged, the emergence time of F1 parasitoids, the longevity of F1 parasitoids, the time to death of F1 parasitoids, and the survival period of F1 parasitoids. Behavioral experiments (trial 1b) were undertaken to identify whether there were any treatment effects on behavior at either trophic level (pest and/or parasitoid), during which both *M. persicae* and parental *A. colemani* behavior were measured.

We also undertook 2 laboratory trials to assess the effects of seed treatments on *M. signatus* (trials 2a and 2b). The traits measured in these trials are shown in Fig. 2 and a schematic outlining the methodology is depicted in Supplementary Fig. S4 for trials 2a and 2b. In trial 2a, undertaken with first- and third-stage *M. signatus*, we measured initial mortality, the longevity of larvae, the number pupating, the number of successful pupations, the total pupation days, and the days to emerge as adults. In trial 2b, undertaken with only first-stage *M. signatus*, we measured initial mortality, the number pupating, and the total pupation days.

Seed Treatments

Untreated ATR Stingray canola seeds were coated with 1 of 3 formulated chemical treatments, using a Hege 11 seed treater (Wintersteiger, Ried im Innkreis, Austria) to produce the following: 600 g/L imidacloprid, at a rate of 400 mL/100 kg (Gaucho 600); 210 g/L thiamethoxam + 37.5 g/L lambda-cyhalothrin, at a rate of 1,000 mL/100 kg (Cruiser Opti); and 350 g/L thiamethoxam, at a rate of 600 mL/100 kg (Cruiser 350FS). These rates reflect the registered field rate of each product in Australian canola (APVMA 2023).

Ten canola seeds of each treatment (in addition to untreated "control" seeds) were planted within plastic pots (100 mm × 100 mm × 75 mm), in an unfertilized, non-sterilized, premium-grade potting mix (Table 1). A total of 24 pots were planted for trial 1a (Supplementary Fig. S1), 36 pots for trial 1b (Supplementary Figs. S2

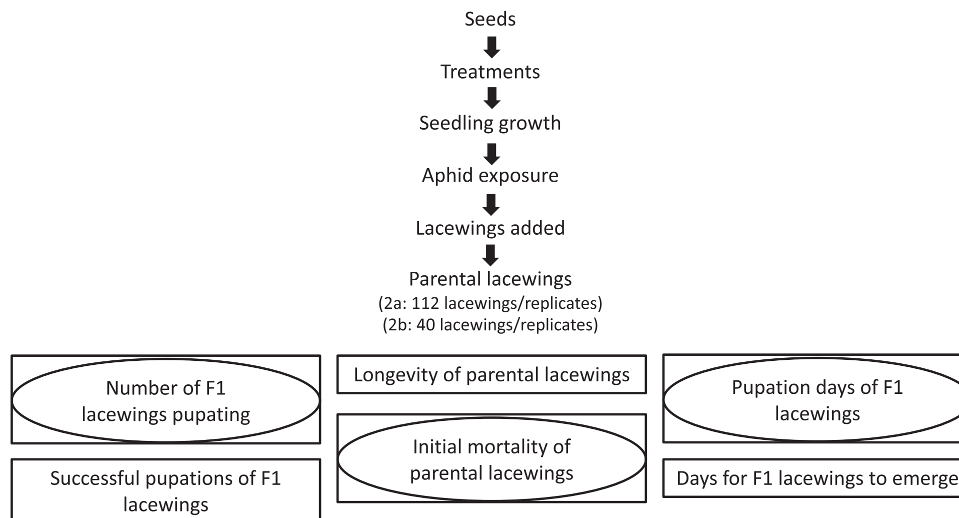

Fig. 2. The traits measured in the *Mallada signatus* experiment (trial 2), with data collected for trial 2a boxed and data collected for trial 2b circled. Replicates for both trials are noted.

Table 1. Canola treatment groups for each trial and number of replicates undertaken, with Insecticide Resistance Action Committee (IRAC) chemical grouping (Sparks and Nauen 2015)

Commercial name	Active ingredient/s	IRAC chemical group	Field product rate	Trial	No. of replicates
Gaucho 600	Imidacloprid	Neonicotinoid (4A)	400 mL/100 kg	1a	6
				1b	12
				2a	7
				2b	10
Cruiser Opti	Thiamethoxam + lambda-cyhalothrin	Neonicotinoid (4A) and pyrethroid (3A)	1,000 mL/100 kg	1a	6
				1b	12
				2a	7
				2b	10
Cruiser 350FS	Thiamethoxam	Neonicotinoid (4A)	600 mL/100 kg	1a	6
				1b	12
				2a	7
				2b	10
Untreated	No chemical treatment	No chemical treatment	No chemical treatment	1a	6
				1b	12
				2a	7
				2b	10

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and S3), 28 pots for trial 2a (Supplementary Fig. S4), and 40 pots for trial 2b (Supplementary Fig. S4). These were placed within a controlled temperature (CT) room at 22°C (±3°C), ~40% relative humidity (RH), and a 16 light (L):8 dark (D) photoperiod. Each pot was placed in a Petri dish and watered sparingly 3 times a week, for 2 weeks. Watering of pots was closely regulated to ensure overwatering did not occur and cause insecticide treatments to leach out of the soil. Each treatment group was placed in a different bug dorm insect rearing cage (4F4590 series, 475 × 475 × 930 mm, Australian Entomological Supplies, Bangalow, New South Wales, Australia) to avoid contamination of insects.

Insects

A population of *M. persicae* was obtained from a tomato crop (*Solanum lycopersicum* L.) in Bendigo, Victoria, Australia (36.565°S, 144.810°E; WGS84) on 3 November 2017. A colony was established in the laboratory on bok choy (*Brassica rapa subsp. chinensis*) after removing parasitoids and generating an isofemale line, to ensure all aphids were of the same haplotype. Subsequent DNA microsatellite analysis of these aphids (see Pym et al. 2022) revealed a single multilocus clone (haplotype 209), which is known to possess resistant alleles for *MACE* and super-*kdr*, amplification of the *E4* esterase gene and an increased copy number of the P450 gene, *CYP6CY3*; these resistance mechanisms have been closely linked to phenotypic resistance to carbamates, pyrethroids, organophosphates, and neonicotinoids, respectively (Bass et al. 2014, de Little et al. 2017, Pym et al. 2022). A resistant *M. persicae* clone was chosen because previous work has shown very high aphid mortality when an insecticide-susceptible clone is exposed to canola seed treatments (Kirkland et al. 2023).

Myzus persicae were maintained on untreated canola plants for 10 generations prior to this study. *Aphidius colemani* (provided by Biological Services, Loxton, South Australia, Australia) were reared on *M. persicae* on canola plants for 2 weeks prior to this study, while *M. signatus* (provided by Bugs for Bugs, Toowoomba, Queensland, Australia) were fed *M. persicae* on canola plants for 4 weeks prior to this study. For both beneficial species, the diet was supplemented with a 20% honey solution, changed weekly. In addition, the *M. signatus* colony was provided with bee pollen from untreated wildflowers (SaxonBee Enterprises, Gidgegannup, Western Australia, Australia). All species were maintained in bug dorm insect rearing cages, within a CT room maintained at 22°C (±3°C), ~60% RH, and a 16L:8D photoperiod.

Experimental Set-Up

Two weeks after sowing, the 3 healthiest canola plants were selected (at the second true leaf stage), and the remaining plants were removed from each pot. Each pot of 3 plants was placed on a Petri dish and separately housed within a sealed plastic microcosm container (102 mm × 108 mm × 200 mm) ventilated with mesh windows. Based on pilot studies, which established the number of aphids that could be placed on plants without leading to plant mortality, ~100 *M. persicae* were then added to each container. Late-stage instars (third- and fourth-stage) and adult apterae were used throughout the trials. All plants continued to be watered sparingly 3 times a week, with water applied directly into the pot so as not to disturb aphids. After 96 h, the aphids were counted for a "day 0" recording.

Trial 1a—*Aphidius colemani*

At day 0, 6 female *A. colemani* were released onto canola plants (infested with *M. persicae*) in each microcosm, after refrigerating at 4°C for 5 min to decrease activity (see Table 1; Fig. 1 and Supplementary Fig. S1). These females were assumed to have mated, as parasitoid sexes were stored together prior to the experiment, with mating usually occurring almost immediately after emergence (Starý 1970). Six microcosms (replicates) were set up per treatment (Fig. 1). The microcosms with parasitoids were held in a CT room at 22°C (±3°C), ~60% RH, and a 16L:8D photoperiod. After 24 h, all living parasitoids were collected from each microcosm and stored in a Petri dish, lined with filter paper and containing a wick dipped in a 20% honey solution (Starý 1970) (Fig. 1 and Supplementary Fig. S1). The duration of seed treatment exposure of the parasitoids was based on similar experiments (e.g., Carter 2013). Wicks in Petri dishes were replaced every 3 days, or more often if dried out or showing signs of mold. Fifty *M. persicae* from the aphid colony that had not been exposed to seed treatments were then added to each Petri dish (Supplementary Fig. S1). Survival of parasitoids within each dish ("Parental parasitoids" in Fig. 1) was recorded daily to assess direct effects of prior exposure to treated aphids within the microcosms. Data analyzed included the initial mortality and longevity of the parental parasitoids (Fig. 1). Petri dishes were checked for aphid mummies 11 days later ("Mummies in Petri dishes" in Fig. 1). Mummies were identified by their engorged, golden/brown appearance (Askew 1971). The proportion of mummies formed was noted and the F1 parasitoids were scored for emergence, longevity, and survival (Fig. 1).

In addition, canola plants on which the parasitoids had originally been added were checked for mummies 10 days after the removal of parental *A. colemani* ("Mummies on plants" in Fig. 1). When present, these mummies were removed from the leaves with a paintbrush (as in Buitenhuis et al. 2005) and all mummies from a microcosm were placed in a single Petri dish for rearing. Petri dishes were checked daily, and the number of mummies formed was counted in addition to the emergence, longevity, and survival of F1 parasitoids being assessed (Fig. 1).

Trial 1b—Aphid and *Aphidius colemani* Behavioral Experiment

These experiments were conducted in a CT room at 22°C (±3°C), ~60% RH, and a 16L:8D photoperiod. For the aphid behavioral experiment, *M. persicae* were maintained on 4 canola plants (1 for each treatment) for 96 h, as described in the "Experimental set-up" section above, after which time aphid numbers were counted to provide a day 0 count. The following day, 12 aphids (replicates) were removed from each treatment and placed within individual Petri dishes under a Leica MS5 microscope mounted with a Leica IC80 HD camera (Supplementary Fig. S2). Each aphid was video recorded for 5 min and their behavior was assessed using traits (inactivity, walking/running, cornicle secretion, movement, and resistance) adapted from Bilodeau et al. (2013) (see Supplementary Table S1; "Aphid behavior" in Fig. 1).

For the parasitoid behavioral experiment, 6 mated female *A. colemani* were released onto canola plants in each microcosm, as in trial 1a (Supplementary Fig. S3). This was repeated 8 times for each seed treatment (32 microcosms in total). From each treatment, 12 parasitoids (replicates) were randomly selected, removed, and placed individually within fresh Petri dishes after 24-h exposure (Supplementary Fig. S3). Along with each parasitoid, 12 naïve aphids (that had not been in contact with parasitoids) from the same chemical treatment (taken from plants in the aphid behavioral experiment) were added to each replicate Petri dish (Table 2; Supplementary Fig. S3). In addition, naïve aphids from untreated plants were also added to

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parasitoids from each chemical treatment and replicated 12 times (Table 2; Supplementary Fig. S3). These treatments in combination provided a test of whether any treatment effects on the interactions between parasitoid and pest was due to the behavior of the aphids or the parasitoids. *Aphidius colemani* were video recorded for 5 min (as described above) and their behavior was assessed using traits (antennal contact, non-oriented contact, resting, oriented walking, cleaning, ovipositor contact A, ovipositor contact B, and searching) adapted from Bilodeau et al. (2013) (see Supplementary Table S1; "Parental parasitoid behavior" in Fig. 1).

Trial 2a—*Mallada signatus* Mixed Life Stages

At day 0, 2 third-stage *M. signatus* larvae and 2 first-stage *M. signatus* larvae were released onto canola plants (infested with *M. persicae*) in microcosm containers (a nested design of replicate lacewings (112 total) nested in 7 replicate microcosms for each of the 4 treatments (28 total); see Table 1; Supplementary Fig. S4). After 24 h, all living lacewings were collected and placed individually within Petri dishes, lined with filter paper, and containing a wick dipped in a 20% honey solution (Supplementary Fig. S4). This duration was chosen to match the exposure time of *A. colemani* within trial 1a and, as for the other trials, this experiment was conducted in a CT room at 22°C (±3°C), ~60% RH, and a 16L:8D photoperiod. Approximately 100 *M. persicae* from the original colony (and not exposed to chemical seed treatments) were added to each Petri dish (Supplementary Fig. S4). These were replenished whenever numbers were depleted. Wicks were replaced every 3 days, or more often if dried out or showing signs of mold. Once in adult form, bee pollen was added to each Petri dish in place of *M. persicae* as a food source. Petri dishes were checked daily for pupation, emergence, longevity, and mortality (Fig. 2). Adult longevity was assessed for up to 120 days.

Trial 2b—*Mallada signatus* First-Stage Larvae

Due to the high mortality of first-stage *M. signatus* larvae in trial 2a, in part due to cannibalism among individuals (which is not uncommon [Duelli 1981]), a second experiment was conducted using a single lacewing per container (40 replicates). This experiment was conducted in a CT room at 22°C (±3°C), ~60% RH, and a 16L:8D photoperiod. At day 0, a single first-stage *M. signatus* larva was placed onto canola plants (infested with 100 *M. persicae*) inside a microcosm container, and this was repeated for each chemical treatment (see Table 1; Supplementary Fig. S4). After 96 h, all living lacewings were collected and placed separately within Petri dishes, lined with filter paper, and containing a wick dipped in a 20% honey solution (Supplementary Fig. S4). Lacewings were kept on the plants for 96 h, as opposed to 24 h in trial 2a, because no significant effects were detected during trial 2a. Approximately 100 *M. persicae* from the original colony (and not exposed to chemical seed treatments) were added to each Petri dish as a food source and were replenished whenever numbers depleted (Supplementary Fig. S4). Wicks were replaced every 3 days, or more often if dried out or showing signs of mold. Petri dishes were checked daily for pupation and mortality (Fig. 2).

Table 2. Summary of treatment combinations explored in the aphid and *Aphidius colemani* behavioral experiments.

Aphid treatment	Parasitoid treatment
Untreated	
Imidacloprid	N/A
Thiamethoxam	N/A
Thiamethoxam + lambda-cyhalothrin	N/A
Untreated	Untreated
Imidacloprid	Imidacloprid
Thiamethoxam	Thiamethoxam
Thiamethoxam + lambda-cyhalothrin	Thiamethoxam + lambda-cyhalothrin
Untreated	Imidacloprid
Untreated	Thiamethoxam
Untreated	Thiamethoxam + lambda-cyhalothrin

Twelve replicates per treatment.

Mass Spectrometry

Mass spectrometry was undertaken on plant material, with 1 sample containing 2 canola cotyledons from each treatment removed on the day of aphid introduction (i.e., 2 weeks post-germination). Mass spectrometry was performed by the Biotechnology and Synthetic Biology Group at the Commonwealth Scientific and Industrial Research Organisation (Black Mountain, Australian Capital Territory, Australia) to confirm the presence (and determine the concentration) of imidacloprid, thiamethoxam, and clothianidin (a metabolite of thiamethoxam [see Nauen et al. 2003, Bredeson et al. 2015]). Plant samples were transported to the facility on dry ice and freeze-dried on arrival.

A Restek LC multiresidue pesticide standard #5 (Restek, 31976; Bellefonte, Pennsylvania, US) was used to make standard curves for imidacloprid, thiamethoxam, and clothianidin. This was initially expressed in parts per billion (ppb), but due to high concentrations, the results were later expressed in parts per million (ppm). The standards were diluted in acetonitrile (Sigma-Aldrich, St. Louis, Missouri, US) and covered 4 orders of magnitude in the range of 0.1–1,000 ppb (covering 0.1, 0.5, 1.0, 5.0, 10, 50, 100, 500, and 1,000 ppb) and were run at the beginning and end of the analysis. The standard curves were linear up to 500 ppb (Supplementary Fig. S5). The slopes, intercept area, and R^2 values of the standard curves for each chemical were as follows: imidacloprid (slope = 0.97, intercept = 7.5, R^2 = 0.998); thiamethoxam (slope = 0.92, intercept = 8.6, R^2 = 0.997); and clothianidin (slope = 0.93, intercept = 7.3, R^2 = 0.998).

Individual plant samples were ground with a 6 mm stainless steel ball in 1 ml of 80% acetonitrile using a Qiagen TissueLyser (Qiagen, Hilden, Germany) at 30 Hz for 3 min, followed by incubation at 4°C for 30 min. Samples were centrifuged to pellet cell debris and the supernatant was applied to an Agilent Captiva EMR Lipid media plate (Agilent, Santa Clara, California, US) to clean the sample from major contaminants. The Captiva EMR Lipid media was previously tested to ensure the compounds of interest were not bound to the Captiva EMR media. The supernatant, 800 µL, was applied to the Captiva EMR media and the recovery of 600 µL was dried down and resuspended in 100 µL prior to analysis using an Agilent 6490 triple quadrupole (QQQ) mass spectrometer. Each neonicotinoid compound was detected within cotyledons that had been treated with their respective chemicals (Table 3).

Lambda-cyhalothrin was purchased from Sigma-Aldrich (St. Louis, Missouri, USA) and included in the mass spectrometry analysis, however, it was unable to be detected in our study. Issues are often associated with the detectability of lambda-cyhalothrin (Gonçalves and Alpendurada 2005).

Data Analysis

All analyses were conducted in R v4.2.1 (R Core Team 2022) except for the Cox regression analyses which were undertaken using Minitab (Minitab 2019). Data were analyzed using Generalized Linear

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Table 3. Concentrations of imidacloprid, thiamethoxam, and clothianidin in canola cotyledons from the various treatments detected through mass spectrometry

		Concentration (ppm)		
Treatment	Weight (mg)	Imidacloprid	Thiamethoxam	Clothianidin
Untreated	2.7	Undetected/trace	Undetected/trace	Undetected/trace
Thiamethoxam	3.4[a]	Undetected/trace	1.706	0.469
Thiamethoxam + lambda-cyhalothrin	3.5	Undetected/trace	0.403	0.086
Imidacloprid	3.3	0.793	Undetected/trace	Undetected/trace

[a]Sample diluted 1/10 prior to analysis.

Models (GLMs). When the response variables were proportions, GLMs were fitted using a quasibinomial distribution with the canonical logit link function, appropriate for values ranging between 0 and 1. When the response variables were counts, GLMs were fitted using a Poisson distribution with the canonical log link function, appropriate for non-negative integers. When the response variables were rates, GLMs were fitted using the inverse Gamma distribution (after adding 1 to all values), appropriate for positive continuous data. Post-hoc Tukey tests for pairwise comparisons of treatment levels were performed using the "emmeans" package v1.8.2 (Lenth et al. 2022). To correct *P*-values for multiple comparisons, a Benjamini–Hochberg false discovery rate (FDR) correction with a 5% threshold was used for all analyses within each trial using the "FDRestimation" package (Murray et al. 2022).

For the parasitoids (trial 1a) we compared treatments for the initial mortality of *A. colemani*, calculated as a proportion of the initial starting population. A Cox regression analysis was undertaken to examine differences between the treatments for parental *A. colemani* survival over time. We compared treatments against mummification rates (on the plants and in Petri dishes), calculated as the proportion of mummies from the total number of aphids per treatment, in addition to the proportion of parasitoids reared from mummies. For the measurements on plants, the number of repeats for the untreated control was reduced from 6 to 5.

Furthermore, we computed emergence times by determining the cumulative days (i.e., ["number of parasitoids emerged on day 1"*1] + ["number of parasitoids emerged on day 2"*2] and so on), with this total then divided by the total number of emerged parasitoids. Average time to death (per replicate) was computed in the same way as emergence times except in this case we counted the cumulative number of parasitoids dying per day, divided by the total number of parasitoid deaths. To investigate the effects on longevity (survival period) of F1 *A. colemani* between plants, the survival period was calculated as the "time to death" subtracted from the "emergence time." GLMs with an inverse Gamma distribution were run to assess the impact of seed treatments for emergence time, time to death, and survival period of parasitoids.

In addition to the above, a total parasitism capability (total number of parasitoids able to parasitize) for *A. colemani* was computed as another way to explore the overall effects of seed treatments. This consisted of a cumulative parasitoid survival period, calculated by the following measure: ("number of emerged parasitoids on day 1"*1) + ("number of emerged parasitoids on day 2"*2) … + ("number of emerged parasitoids on day 11"*11). This measure incorporated counts of all parental *A. colemani*, F1 *A. colemani* produced on the untreated or treated plants, and F1 *A. colemani* produced within the Petri dishes. This is important from a biological control perspective because it provides a population-wide measure of the number of parasitoids available to parasitize aphids. A GLM was run to assess the total parasitism capability.

For the behavioral assessments (trial 1b) of *M. persicae*, the effects of the 4 treatments were compared. For the behavioral assessments of *A. colemani*, the effects of the 7 combinations of seed treatment exposure were compared, with behaviors displayed by both *M. persicae* and *A. colemani* compared against treatments.

For *M. signatus*, GLMs were undertaken to analyze initial mortality, the number of pupations, the number of successful lacewing pupations, adult longevity, emergence time, and pupal duration in trial 2a. For trial 2b, the number of pupations was analyzed with a χ^2 goodness-of-fit test and pupal duration was also analyzed with a GLM.

Results

Aphidius colemani

Trial 1a—Survival of parental parasitoids.

Parental *A. colemani* survival (number of parasitoids alive) after 24 h did not significantly differ between the seed treatments and untreated controls (GLM, quasibinomial family, logit link, $F_{3,20} = 0.33$; $P = 0.801$). In addition, a Cox regression analysis indicated no significant difference between the treatments for parental *A. colemani* survival (number of parasitoids alive versus days passed) throughout the trial ($F_{3,140} = 0.19$, $P = 0.903$, $R^2 = 0.41\%$; Fig. 3).

Trial 1a—Mummies collected from plants.

Fewer aphid mummies were produced on the untreated canola, resulting in an overall treatment effect (GLM, quasibinomial family, logit link, $F_{3,19} = 10.52$, $P < 0.001$; Fig. 4). Post-hoc tests indicate the number of mummies formed on untreated plants and on thiamethoxam treated plants were significantly lower than those formed on imidacloprid- and thiamethoxam + lambda-cyhalothrin-treated plants (Fig. 4).

The proportion of *A. colemani* reared from mummies (i.e., F1 parasitoids) was also affected by treatment (GLM, quasibinomial family, logit link, $F_{3,19} = 8.44$, $P = 0.001$), with a higher proportion of individuals reared on the thiamethoxam + lambda-cyhalothrin plants compared with the thiamethoxam and untreated plants (Fig. 5).

No significant treatment effect was identified for emergence times (GLM, inverse Gamma family, $F_{3,19} = 0.27$, $P = 0.845$), time to death (GLM, inverse Gamma family, $F_{3,19} = 1.28$, $P = 0.311$), or survival periods (GLM, inverse Gamma family, $F_{3,19} = 1.91$, $P = 0.163$) of F1 *A. colemani* collected as mummies from plants.

Trial 1a—Mummies collected from Petri dishes.

No significant difference among treatments was detected for the proportion of aphid mummies formed (GLM, quasibinomial

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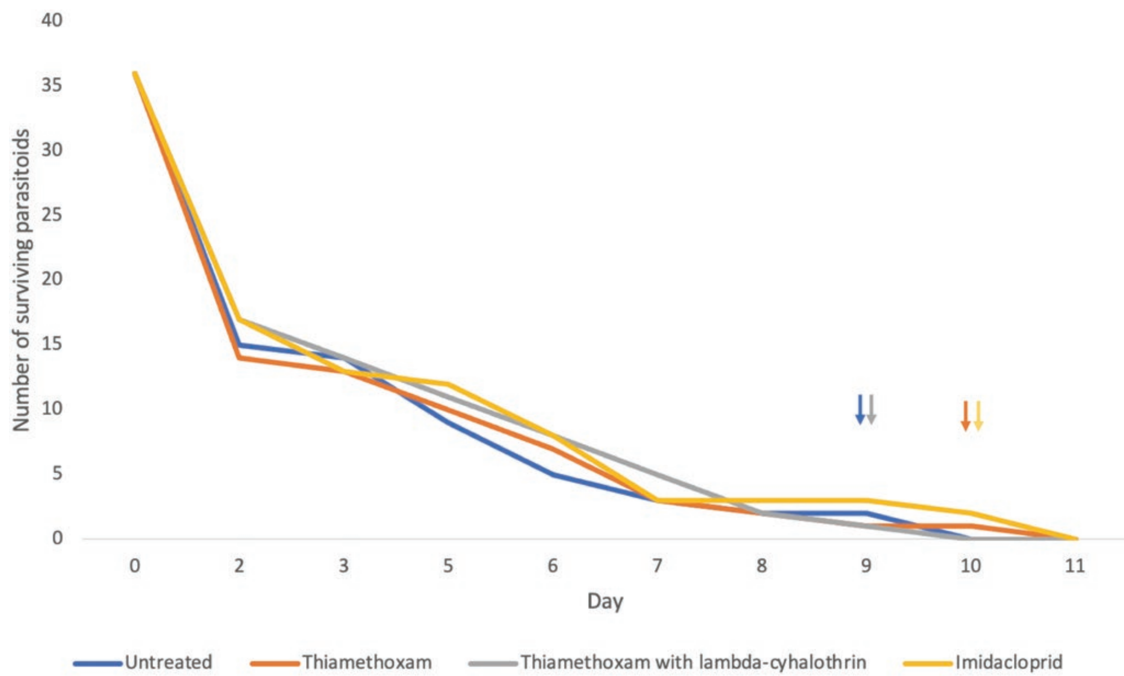

Fig. 3. *Aphidius colemani* survival with different seed treatments (arrows indicate last day of parasitoid survival for the color-coded treatment).

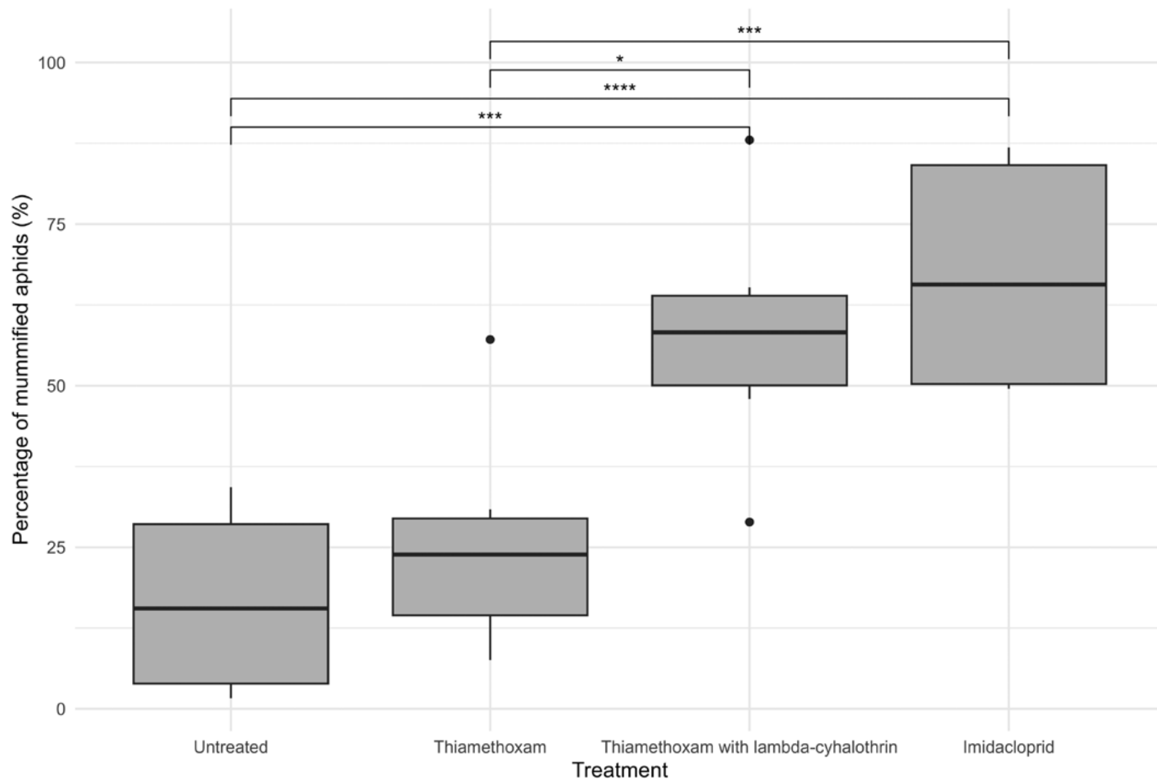

Fig. 4. A box plot depicting the percentage of mummified aphids formed from total aphids on untreated, and seed-treated plants. Points represent outliers. Asterisks indicate statistical differences between treatments from post hoc comparisons (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$; and **** $P < 0.001$).

family, logit link, $F_{3,59} = 0.84$, $P = 0.479$) or the proportion of F1 *A. colemani* reared from mummies (GLM, quasibinomial family, logit link, $F_{3,20} = 1.31$, $P = 0.299$) within the Petri dishes. Similar to F1 *A. colemani* collected from plants, the emergence times (GLM, inverse Gamma family, $F_{3,20} = 0.38$, $P = 0.768$), time to death (GLM, inverse Gamma family, $F_{3,20} = 0.40$, $P = 0.752$), and survival period (GLM, inverse Gamma family, $F_{3,20} = 0.93$, $P = 0.445$) of F1 *A. colemani* within the Petri dishes were not significantly different between treatments.

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Fig. 5. A box plot depicting the percentage of F1 *Aphidius colemani* reared from total mummies on untreated and seed treated plants. Points represent outliers. Asterisks indicate statistical differences between treatments from post hoc comparisons (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$; and **** $P < 0.001$).

***Trial 1a—Population fitness of* Aphidius colemani.**

As an overall measure of population fitness, the cumulative survival days across generations were estimated for *A. colemani*. This measurement was significantly different between treatments (GLM, Poisson family, log link, $F_{3,19} = 4.47$, $P = 0.016$). All pairwise comparisons were statistically significant; the fitness of *A. colemani* was highest in the thiamethoxam + lambda-cyhalothrin treatment, followed by the imidacloprid treatment, followed by the thiamethoxam treatment, and lowest in the untreated controls (Fig. 6), although this effect was no longer significant after using a Benjamini–Hochberg FDR correction with a false discovery rate of 5% ($P = 0.062$).

Trial 1b—Behavioral experiments.

There were no treatment effects for *M. persicae* behavior observed in trial 1b. The periods *M. persicae* spent inactive (GLM, Poisson family, log link, $F_{3,44} = 0.59$, $P = 0.624$), walking (GLM, Poisson family, log link, $F_{3,44} = 0.59$, $P = 0.624$), or showing any signs of other movement (GLM, Poisson family, log link, $F_{3,44} = 0.92$, $P = 0.441$) did not significantly differ between treatments. When in the presence of *A. colemani*, there were also no treatment effects observed in *M. persicae* behavior; there were no significant differences between treatments for the number of kicks by an aphid when attacked by a parasitoid (based on any ovipositor contact) (GLM, Poisson family, log link, $F_{6,77} = 1.73$, $P = 0.126$) or the total number of kicks as a proportion of the number of attacks (ovipositor contact A and B) (GLM, Poisson family, log link, $F_{4,12} = 1.99$, $P = 0.161$).

There were, however, some treatment effects on *A. colemani* behavior. The duration of cleaning and searching by *A. colemani* was significantly different between treatments (cleaning: GLM, Poisson family, log link, $F_{6,77} = 5.02$, $P < 0.001$; searching: GLM, Poisson family, log link, $F_{6,77} = 5.41$, $P < 0.001$) (Fig. 7) but not of oriented walking (GLM, Poisson family, log link, $F_{6,77} = 1.15$, $P = 0.340$). When *A. colemani* were paired with aphids from the same treatments, *A. colemani* on imidacloprid-treated plants spent less time searching than those from the other treatments, and *A. colemani* on the thiamethoxam-treated plants spent less time cleaning than those from the other treatments. When *A. colemani* from untreated plants were paired with *M. persicae* from insecticide seed-treated plants, those paired with aphids from the thiamethoxam + lambda-cyhalothrin treatment spent the most time cleaning and the least time searching, and those paired with aphids from imidacloprid-treated plants the least time cleaning and the most time searching (Fig. 7). Other *A. colemani* behaviors were not significantly different between treatments when analyzed by GLMs with a Poisson family and a log link function: duration of ovipositor contact (A, $F_{6,77} = 1.61$, $P = 0.155$; B, $F_{6,77} = 1.32$, $P = 0.257$; total, $F_{6,77} = 1.52$, $P = 0.183$); antennal contact ($F_{6,77} = 1.61$, $P = 0.155$); and resting time ($F_{6,77} = 1.64$, $P = 0.148$).

Mallada signatus

Trial 2a—Pupation, emergence, longevity, and mortality.

No significant differences were found between treatments for each of the variables tested against *M. signatus* in trial 2a. When treatments were combined, initial mortality for the first-stage larvae equated to an average of 39% but for the third-stage larvae it was lower at 14%. There was no significant treatment effect on other traits (all compared with GLMs with a quasibinomial family and a logit link function): the initial mortality of first-stage lacewing larvae ($F_{3,24} = 0.33$, $P = 0.801$); third-stage lacewing larvae ($F_{3,24} = 1.41$, $P = 0.264$); and total lacewing larvae ($F_{3,24} = 1.53$, $P = 0.232$).

There were no treatment effects on the number of total lacewings pupating (first-stage larvae: GLM, quasibinomial family, logit link, $F_{3,24} = 0.62$, $P = 0.606$; third-stage larvae: GLM, quasibinomial family, logit link, $F_{3,24} = 1.64$, $P = 0.205$; total lacewings: GLM,

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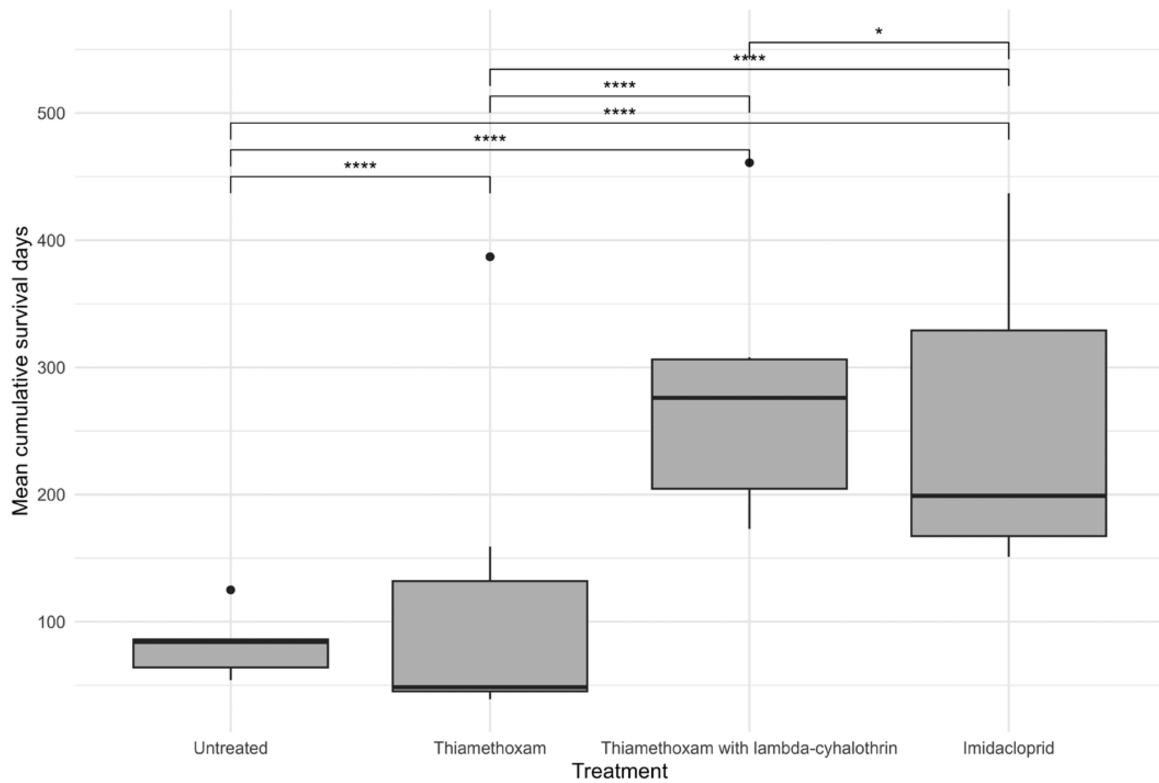

Fig. 6. A box plot depicting the total parasitism capability for *Aphidius colemani* on untreated and seed-treated plants, as per mean cumulative survival days. Points represent outliers. Asterisks indicate statistical differences between treatments from post hoc comparisons (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$; and **** $P < 0.001$).

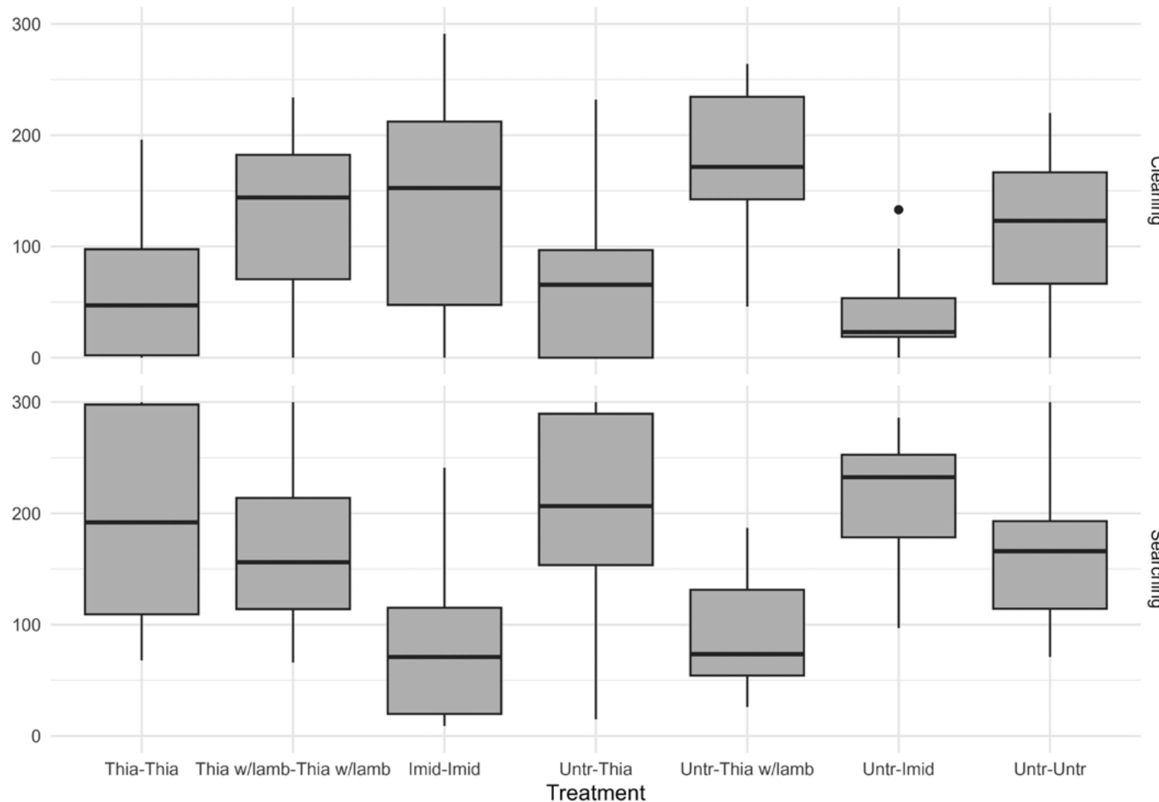

Fig. 7. Box plots depicting the treatment effects on time spent by *Aphidius colemani* a) cleaning and b) searching. Points represent outliers. ("Treatment" indicates first the parasitoid treatment and second the paired *Myzus persicae* treatment; "Imid" = Imidacloprid, "Thia w/lamb" =Thiamethoxam with lambda-cyhalothrin, "Thia" =Thiamethoxam, and "Untr" = Untreated).

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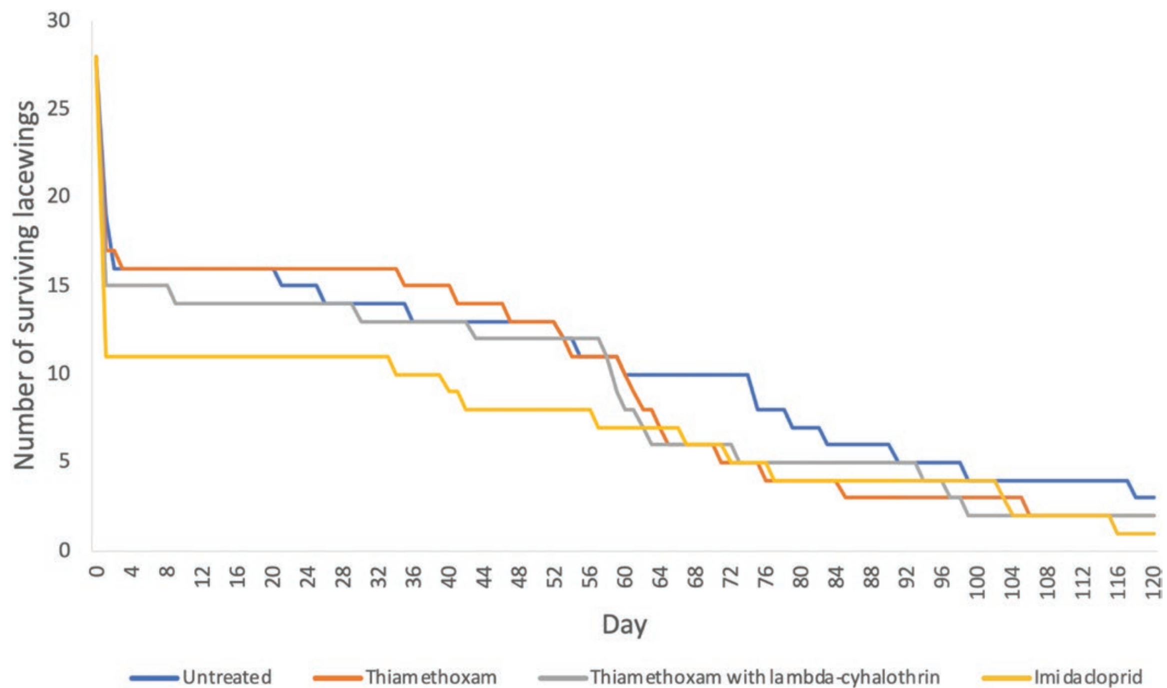

Fig. 8. *Mallada signatus* survival when exposed to *Myzus persicae* feeding on canola plants treated with different insecticides.

quasibinomial family, logit link, $F_{3,24} = 0.85$, $P = 0.480$). The pupation days of F1 lacewings did not significantly vary across treatments: first-stage larvae (GLM, Poisson family, log link, $F_{3,26} = 0.10$, $P = 0.961$); third-stage larvae (GLM, Poisson family, log link, $F_{3,35} = 0.12$, $P = 0.945$); and total lacewings (GLM, Poisson family, log link, $F_{3,65} = 0.18$, $P = 0.911$). First-stage larvae had an average total pupation success of 45% and the third-stage larvae had an average pupation success of 61%. The proportion of successful pupations did not differ significantly between treatments (GLM, quasibinomial family, logit link, $F_{3,24} = 2.54$, $P = 0.080$). The total number of days for lacewing larvae to emerge from pupae did not differ significantly between treatments (GLM, Poisson family, log link, $F_{3,55} = 2.20$, $P = 0.099$).

There was no significant difference in adult longevity of *M. signatus* (from pupal emergence to death) between treatments (GLM, Poisson family, log link, $F_{3,57} = 0.18$, $P = 0.911$) (Fig. 8).

Trial 2b—Pupation.

In trial 2b, there were no significant differences between treatments in the number of lacewings pupating ($\chi^2 = 0.77$, $df = 3$, $P = 0.856$) or the number of days taken for *M. signatus* to pupate (GLM, Poisson family, log link, $F_{3,31} = 1.06$, $P = 0.379$). All pupations were successful. At trial completion, only 2 lacewings had died, 1 in the untreated control and 1 in the imidacloprid treatment.

Discussion

Neonicotinoids, as highly water-soluble chemicals, are transported to different plant tissues through water movement from the xylem, the phloem, or both (Bonmatin et al. 2015). These chemicals (or their metabolites) persist in the plant for some time, although this can vary across neonicotinoid and plant types (Alford and Krupke 2017), and the length of toxicity can vary depending on insect species (Kirkland et al. 2018). In addition to the pest species being targeted, many studies have shown neonicotinoids to be toxic to parasitoids and predators. For example, when natural enemies were exposed through tri-trophic interactions to insecticide seed treatments, Gontijo et al. (2018) found thiamethoxam caused mortality of the stink bug, *Podisus nigrispinus* (Dallas), a predator of the fall armyworm *Spodoptera frugiperda* (Smith). Douglas et al. (2015) found that although the pest slug *Deroceras reticulatum* (Müller) was unaffected by thiamethoxam, the chemical was passed to the predatory beetle, *Chlaenius tricolor* Bonelli, which fed on the slugs, impairing or killing > 60% of the beetles. In another study, Naveed et al. (2010) found lower field parasitism rates of the whitefly *Bemisia tabaci* (Gennadius) by aphelinid parasitoids when cotton seeds were treated with thiamethoxam and imidacloprid. We, therefore, expected the seed treatments tested here to have negative effects on *A. colemani* and *M. signatus,* both important beneficial insects in Australian canola, yet this study found no strong evidence for such effects.

Aphidius colemani

Aphidiines feed on honeydew produced by aphids (Wäckers 2005) and a number of studies show contaminated honeydew can be toxic to parasitoids (Calvo-Agudo et al. 2019, 2020, 2021, 2022, Quesada et al. 2020). For example, consumption of honeydew from soybean aphids (*Aphis glycines* Matsumura) that fed on soybean plants grown from neonicotinoid-coated seeds was found to reduce the longevity of the parasitoid wasp *Aphelinus certus* Yasnosh (Calvo-Agudo et al. 2021). Furthermore, there are records of imidacloprid detection within the honeydew of the striped pine scale *Toumeyella pini* (King) (Quesada et al. 2020), glucosinate sinigrin detection within the honeydew of *M. persicae* (Merritt 1996), and terpenoid detection within the honeydew of *Aphis gossypii* Glover (Hagenbucher et al. 2014). However, this potential exposure pathway can be influenced by the toxins to which the host aphid is exposed. A study by Nauen (1995) determined that low concentrations of imidacloprid strongly depressed the honeydew excretion of apterous *M. persicae* adults by almost 95% within 24 h. In future studies, we recommend both the

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volume of honeydew produced and the insecticide levels within the honeydew of aphids be tested. In the current study, very little honeydew was present for the natural enemies to feed on, suggesting limited exposure through this pathway.

There were differences in the number of aphid mummies produced on plants, with the number of mummies formed on untreated plants and on thiamethoxam-treated plants significantly lower than those formed on thiamethoxam + lambda-cyhalothrin- and imidacloprid-treated plants. Furthermore, a higher proportion of F1 *A. colemani* was reared on the thiamethoxam + lambda-cyhalothrin plants compared with the thiamethoxam and untreated plants. It is interesting that the 2 seed treatments containing thiamethoxam vary so dramatically in these measurements. Although thiamethoxam was present at the same rate within both the seed treatments, the mass spectrometry results suggest a lower uptake of thiamethoxam in plants that were treated with thiamethoxam in combination with lambda-cyhalothrin. *Aphidius colemani* exposed to the thiamethoxam + lambda-cyhalothrin treatment had a higher fitness than those exposed to the thiamethoxam-only treatment. The presence of lambda-cyhalothrin could potentially interact metabolically within the plant and/or aphid, resulting in lower levels of the neonicotinoid reaching the parasitoids. For example, in cotton leaves, the half-life of thiamethoxam is 1.9 days when present by itself but is reduced to 1.6 days when in combination with lambda-cyhalothrin (Xuyang et al. 2013).

But why did we observe an apparent benefit of some insecticide seed treatments on *A. colemani* rather than negative effects as predicted? The mass spectrometry results confirmed the chemicals were taken up by the canola plants, and the levels of each compound were similar or greater than those reported in other studies (e.g., see Krischik et al. 2015). Our findings might be explained by a change in host behavior, with the aphid becoming less fit and therefore less able to defend itself from attack (Booth et al. 2007). *Aphidius colemani* from the imidacloprid treatment spent the shortest amount of time searching for same-treatment hosts than parasitoids from the other treatments. However, when *A. colemani* from the untreated plants were provided with *M. persicae* from the chemical seed treatments, those paired with aphids from the thiamethoxam + lambda-cyhalothrin treatment spent the least amount of time searching and the most time cleaning, and those paired with aphids from imidacloprid treated plants the most time searching and the least time cleaning. This could suggest lambda-cyhalothrin and/or thiamethoxam (although not the case in the thiamethoxam-only treatment) inhibited the ability of *A. colemani* to locate *M. persicae*. This phenomenon has been noted by Mustard et al. (2020), who determined thiamethoxam directly affected the olfactory perception of odors and the foraging ability of honeybees (*Apis mellifera* L.). In addition, lambda-cyhalothrin can impair orientation in *Aphidius ervi* (Haliday), a parasitoid of *M. persicae* (Desneux et al. 2004).

Another explanation could be due to hormoligosis. Many studies have shown hormoligosis in insects, a phenomenon that predicts that sub-harmful levels of an insecticide will be stimulatory to an organism through the provision of increased efficiency and increased sensitivity to respond to environmental changes (Luckey 1968). Cutler (2013) lists several studies where increased fecundity, stimulated oviposition, and decreased pupal mortality have been reported. One such study found that DDT injected into a braconid parasitoid stimulated oviposition (Grosch and Valcovic 1967). In our study, significant effects of seed treatments were constrained to the F1 *A. colemani* produced on the insecticide seed-treated plants. This suggests that any treatment effects on *A. colemani* may not be long-lasting. This is congruent with other studies involving insecticide exposure. For example, Nauen (1995) found that within 24 h of being removed from imidacloprid-treated leaves, *M. persicae* reversed their immediate behavioral responses and began increasing in weight and producing more honeydew. Furthermore, *A. ervi* orientation and oviposition behaviors towards *M. persicae* that were found to be impaired by lambda-cyhalothrin dissipated after 24 h (Desneux et al. 2004).

Mallada signatus

No significant effects of insecticide seed treatments were found on *M. signatus* mortality, larval and pupal survival, larval and pupal duration, or adult longevity. Other studies have found lacewings to be tolerant to a range of agricultural insecticides. For example, imidacloprid was shown to have low toxicity to the green lacewing *Chrysoperla rufilabris* (Burmeister), causing 1–11% mortality (Mizell III and Sconyers 1992). A field study in sorghum crops also indicated imidacloprid seed treatments to have little to no impact on lacewings, but negatively affect other predators such as the ladybird beetle *Hippodamia convergens* Guerin (Krauter et al. 2001). Conversely, a study in cotton found that exposure of the larvae and adults of the green lacewing, *Chrysoperla externa* (Hagen), to thiamethoxam-treated plants caused sub-lethal and transgenerational effects (Sâmia et al. 2019). Gontijo et al. (2014) also explored the impacts of thiamethoxam-treated sunflower seeds on the green lacewing *Chrysoperla carnea* and found it to be toxic, reducing the fecundity and survival of adults. The number of *Chrysoperla* sp. adults was also reduced in soybean fields grown from thiamethoxam-treated seed (Seagraves and Lundgren 2012). Gontijo et al. (2014) suggest the greater impact of seed treatments on adult lacewings may be, in part, due to their greater consumption of extra-floral nectar. Thus, the lack of significant effects in our study could be due to a lack of extra-floral nectar feeding, given the canola plants used here were not at the flowering stage. Juveniles were fed contaminated aphids, but after pupation, the adults were fed untainted pollen.

Selectivity of insecticides to beneficial organisms is important for the implementation of integrated pest management programs and for conservation biological control (Sterk et al. 1999, Jansen et al. 2008, Bacci et al. 2009). Surprisingly, little evidence of negative toxic effects against aphid natural enemies was detected here. Although unexpected, our results are similar to findings from some other studies. It is possible the initial exposure time to chemicals in our study may have been insufficient to trigger the full extent of lethal or sublethal effects against *M. signatus* and *A. colemani*. Survival of the parasitoid, *Lysiphlebus testaceipes* Cresson, after exposure to azadirachtin, was found to significantly reduce from 69% to 80% at 24 h after treatment to 28–33% at 48 h after treatment (Tang et al. 2002). In a study undertaken by Anjum and Wright (2016), the intrinsic toxicity of lambda-cyhalothrin was greater against *M. persicae* when exposure time increased from 24 to 120 h. These exposure time comparisons suggest the results we observed might have been different if the exposure time of aphids (and/or natural enemies) had been extended (Bostanian et al. 2005). This could be investigated through further experimentation. It is also worth noting that the toxicity of insecticides can vary between related species (Prabhaker et al. 2007, Wang et al. 2013, Overton et al. 2021), and between different populations of the same species (Huseth et al. 2016). In this study, we used a single colony of each natural enemy, both of which had been in laboratory cultures for some time. Further experiments should ideally be undertaken to investigate other populations of these species, and other closely related

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species, including those collected directly from the field, to determine how widespread these patterns are across and within taxa. It is also important for future trials to be undertaken using semi-field or field studies, given that chemical impacts can vary considerably with laboratory trials such as those conducted here (Hassan et al. 1988). Many factors may influence the dissipation rate of seed treatments such as temperature, precipitation, moisture content, sunlight, and response mechanisms of target plants (Fantke and Juraske 2013, Jiang et al. 2019); these factors cannot be easily replicated in the laboratory. In the field, insects will also be exposed to other insecticides and pathogens, with interactions between these stressors and insecticide exposure potentially affecting survival and fitness (Doublet et al. 2015, Grassl et al. 2018).

Now commonplace in canola crops globally, insecticide seed treatments are typically considered a "softer" option to foliar sprays, yet there are variable results on the effects of such seed treatments when it comes to natural enemies. Our study on *A. colemani* and *M. signatus* found little evidence of negative toxic effects (both lethal and sublethal) and where these were identified they were relatively short-lived. Furthermore, in the case of *A. colemani,* exposure to some seed treatments led to an increase in mummification rate and parasitoid emergence, perhaps due to hormoligosis. These results point to the complexity of ecotoxicology studies involving multiple trophic levels and indicate that seed treatments may have variable impacts on key fitness traits of natural enemies.

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Author contributions

Samantha Ward (Conceptualization [equal], Data curation [lead], Formal analysis [equal], Investigation [lead], Methodology [equal], Validation [lead], Writing—original draft [lead], Writing—review & editing [equal]), Ary Hoffmann (Conceptualization [equal], Formal analysis [equal], Funding acquisition [equal], Supervision [lead], Writing—review & editing [equal]), Maarten Van Helden (Conceptualization [equal], Writing—review & editing [equal]), Alex Slavenko (Formal analysis [supporting]), and Paul Umina (Conceptualization [equal], Funding acquisition [equal], Supervision [supporting], Writing—review & editing [equal])

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Supplementary material

Supplementary material is available at *Journal of Economic Entomology* online.

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