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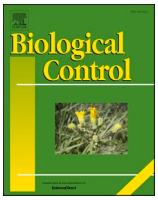

Effect of *Rickettsiella viridis* endosymbionts introduced into *Myzus persicae* aphids on parasitism by *Diaeretiella rapae*: A combined strategy for aphid control?

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HIGHLIGHTS

- Female parasitoid *Diaeretiella rapae* prefer to probe *Myzus persicae* aphids transinfected with the endosymbiont *Rickettsiella viridis.*
- Infection with *R. viridis* does not provide *M. persicae* with protection against *D. rapae*.
- Consistent with earlier research, *R. viridis* can spread among *M. persicae* populations despite significant fitness costs.
- While plant-based transmission is likely the most important factor in the spread of *R. viridis*, horizontal transmission via parasitoids may also contribute.
- Simultaneous release of *M. persicae* infected with *R. viridis* and *D. rapae* early in the growing season could help biological control of this aphid.

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ABSTRACT

Aphids are major crop pests in southeastern regions of Australia. Some aphid species harbor heritable facultative endosymbionts that may induce beneficial or detrimental impacts on aphids under certain ecological conditions. Aphid-parasitoid interactions can be greatly affected by facultative endosymbionts but there is still limited research on many species of economic significance. Here we assessed the effects of a facultative endosymbiont, *Ricketsiella viridis,* on parasitism of the major aphid pest, *Myzus persicae,* by *Diaeretiella rapae*. We found that *R. viridis* does not provide *M. persicae* with significant protection against *D. rapae*, with parasitoids showing a preference for probing aphids infected with *R. viridis*. The fecundity of *M. persicae* is reduced due to infection with *R. viridis* regardless of the presence of parasitoids. Moreover, we show that parasitoids may facilitate horizontal and subsequent vertical transmission of facultative endosymbionts in aphids which could increase the spread of deleterious effects associated with *R. viridis*. Based on these findings, simultaneous release of *D. rapae* and *M. persicae* infected with *R. viridis* in the early cropping season (lower population densities and cooler conditions) may contribute to an effective strategy for efficient management of this pest.

1. Introduction

The peach potato or green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) is a major pest throughout the world (Van Emden and Harrington, 2017) which feeds on and damages plants of over 40 families, particularly *Brassica* L. species in temperate regions (Cole, 1997), and transmits over 100 plant-infecting viruses (Blackman and Eastop, 2000). Most grain crop yield loss due to *M. persicae* is attributed to virus transmission, including the economically important virus of canola, Turnip Yellows virus (TuYV; Family Luteoviridae, Genus Polerovirus) (Congdon et al., 2020). TuYV is persistently aphid-transmitted (circulative and non-propagative) and host phloem limited (Schliephake, E et al., 2000). Chemical control is the main approach for reducing the direct and indirect impacts of pest aphids (DeBach and Rosen, 1991; Desneux et al., 2004; Gullan and Cranston, 2014; Hardin et al., 1995). However, considering the harmful effects of exposure to chemicals on the whole ecosystem and the fact that *M. persicae* has evolved resistance to more than 70 different insecticides (Bass et al.,

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2014; De Little et al., 2017; Umina et al., 2014), biological control strategies need to be developed against this pest. *Diaeretiella rapae* (McIntosh) (Hymenoptera: Aphidiidae) is an important biological control agent of aphids feeding on Brassicaceae (Blande et al., 2004), including *M. persicae* elio (Elliott et al., 2014; Pike et al., 1999) in both the laboratory (Silva et al., 2011) and in field crops (Ward et al., 2021). The success of biological control of *M. persicae* is heavily reliant on the life history strategies of both interacting insects (Khatri et al., 2017).

Aphids have established long-term mutualisms with obligate microbial symbionts, the most common being *Buchnera aphidicola*, an endosymbiont which provides them with essential amino acids lacking in plant phloem (Baumann, 2005; Douglas, 1989; Guo et al., 2017; Shigenobu et al., 2000). Aphids also harbor other heritable facultative endosymbionts that may induce either beneficial or detrimental effects on aphids under certain ecological conditions (Oliver et al., 2010). These endosymbionts may confer better performance on host plants, heat tolerance, protection against natural enemies and body color changes, but can also have fitness costs in some circumstances (Asplen et al., 2014; Peccoud et al., 2015). Aphid-parasitoid interactions can be greatly affected by facultative endosymbionts (Oliver et al., 2010). Aphids harboring *Hamiltonella defensa* (γ-Proteobacterium) show resistance against parasitoid wasps that lay eggs inside their hosts (Brandt et al., 2017; Oliver et al., 2003; Vorburger et al., 2003). Likewise, aphids infected by *Rickettsiella viridis* and *H. defensa* may show altered survivorship when confronting predators such as ladybeetles (Polin et al., 2015). Furthermore, parasitoids can facilitate horizontal transmission between aphids by probing infected and uninfected individuals, acting as vectors to promote the spread of endosymbionts (Russell et al., 2003; Russell and Moran, 2005).

The gamma proteobacterial facultative symbiont, *Rickettsiella viridis,* occurs naturally in *Acyrthociphon pisum* and can be found in host hemolymph, sheath cells, secondary bacteriocytes and other tissues (Tsuchida et al., 2010). Infection with *R. viridis* changes *A. pisum* body color from red to green (Tsuchida et al., 2010) and may provide them with context-dependent protection against predators (Polin et al., 2015). *Rickettsiella viridis* has recently been introduced into a non-native host, *M. persicae,* where it induces similar body color changes (Gu et al., 2023). There are fitness costs associated with *R. viridis* infection in both *A. pisum* (Tsuchida et al., 2014) and *M. persicae*, where it reduces fecundity and thermal tolerance (Gu et al., 2023). Since other endosymbionts can provide parasitoid protection and since aphid body color can influence parasitoid preferences (Libbrecht et al., 2007) *R. viridis* infection may also influence aphid-parasitoid interactions. However, while *R. viridis* protects aphids against the entomopathogenic fungus *Pandora neoaphidis* (Łukasik et al., 2013), no studies to date have tested whether *R. viridis* can provide protection against parasitoids.

The release of *R. viridis* and other endosymbionts in the field may provide a way of modifying aphid populations by introducing fitness costs and thereby reducing plant damage (Gu et al., 2023). Here we investigated the effects of *R. viridis* on parasitism by *D. rapae* using aninfected line of *M. persicae* developed by Gu et al. (2023). We then examined whether *R. viridis* affected selection of hosts for parasitism by *D. rapae* when given a choice of infected and uninfected *M. persicae*. Additionally, we evaluated whether there was horizontal transmission of *R. viridis* via *D. rapae*. Finally, we tracked the spread of *R. viridis* in the presence or absence of parasitoids at cool temperatures in the laboratory. These results help to predict population dynamics of *R. viridis* in the field which will dictate its usefulness as a biocontrol agent.

2. Material and methods

2.1. M. persicae lines and maintenance

All experiments were conducted using a single clone of *M. persicae* with the multilocus genotype 188, except for the horizontal transmission experiment where two additional clones with the multilocus genotypes of Kyabram 98 and Elliott 158 (Umina et al., 2022) were used to test for *R. viridis* transmission via *D. rapae*. The Grains Innovation Park in Horsham, Victoria provided genotype 188, while multilocus genotype Kyabram 98 was collected from Victoria (GPS: −36.383, 145.033) in April 2002 and multilocus genotype Elliotte 158 was collected from Queensland (GPS: −24.982, 152.304) in October 2017 (Umina et al., 2022). Multilocus genotype 188 from super clone B has resistance to carbonate, synthetic pyrethroids, organophosphates, and some resistance to neonicotinoids, whereas the resistance status of multilocus genotypes Kyabram 98 and Elliotte 158 are unknown (Gu et al., 2023). These genotypes, along with others, can be differentiated using a set of microsatellite loci (Umina et al., 2022). The main purpose of using these two multilocus genotypes was to ensure that positive detections of *R. viridis* in aphids in transmission experiments were not due to contamination with aphids infected with *R. viridis* (designated R+) from the initially infected clone.

The facultative endosymbiont *R. viridis* had previously been introduced via microinjection to genotype 188 aphids from an *A. pisum* donor which was originally infected with *R.viridis* (Yang et al., 2023), collected on lucerne (*Medicago sativa*) from Tintinara, South Australia in 2019, with hemolymph of the donor injected into *M. persicae* nymphs (Gu et al., 2023). A clonal line of *M. persicae* infected with *R. viridis* (R+) was established and maintained under laboratory conditions on bok choy (*Brassica rapa* subsp. *chinensis*; grown at ambient conditions in a shade house). All aphids were bulk cultured three weeks prior to experiments in insect rearing cages (30 × 30 × 62 cm, mesh 160 μm aperture to prevent escaping of aphids) on whole bok choy plants in controlled temperature (CT) cabinets at 18 °C and a photoperiod of 16L:8D (Gu et al., 2023). We synchronized aphids at desired nymphal stages by placing 20 adults of *M. persicae* on bok choy leaf discs (35 mm diameter) in petri dishes (60 × 15 mm) covered by a base layer of 1% agar to maintain high humidity. Petri dishes were kept in CT cabinets, where we collected nymphs from each petri dish daily and reared them in new petri dishes under the same conditions to obtain nymphs of the desired stage.

2.2. Facultative endosymbiont screening

We used 150 μL of 5% Chelex 100 Resin (Bio-Rad laboratories, Hercules, California, USA) and 3 μL of Proteinase K (20 mg/ mL) (Roche Diagnostics Australia, Castle Hill, New South Wales, Australia) to extract genomic DNA from insects. Extracted DNA was incubated for 30 min at 65 °C followed by 10 min at 90 °C. A 1 in 3 dilution of pure DNA was used to screen for *R. viridis* and its relative density with a RT-qPCR (Roche Light Cycler 480) assay (Lee et al., 2012). DNA amplification for RT-qPCR began with a 10 min pre-incubation at 95 °C (Ramp Rate = 4.8 °C/s), then 40 cycles of 95 °C for 5 sec. (Ramp Rate = 4.8 °C/s), 65 °C for 15 sec. (Ramp Rate = 2.5 °C/s) and 72 °C for 30 sec. (Ramp Rate = 4.8 °C/s) (Chirgwin et al., 2022).

We used two primer sets to confirm the presence of aphid DNA (β-actin as reference gene; F: GTGATGGTGTATCTCACACTGTC and R: AGCAGTGGTGGTGAAACTG) and *R. viridis* (RCL16S-211; F: GGGCCTTGCGCTCTAGGT and R: TGGGTACCGTCACAGTAATCGA). Crossing point (Cp) values of three consistent replicates were averaged and the differences between the mean Cp values of the *actin* reference gene and *R. viridis* markers were used to calculate ΔCp values. Standard deviations (SD) were measured based on ΔCp values of the technical replicates. Technical replicates with SD < 0.5 were considered valid and ΔCp values were transformed by 2^n to produce relative endosymbiont density (Chirgwin et al., 2022).

2.3. Diaeretiella rapae line and maintenance

Biological Services (Loxton, South Australia) supplied mummies of *D. rapae* collected from *Lipaphis pseudobrassicae* (Turnip aphid) infesting bok choy. *Diaeretiella rapae* were kept in CT cabinets at 18 °C and a

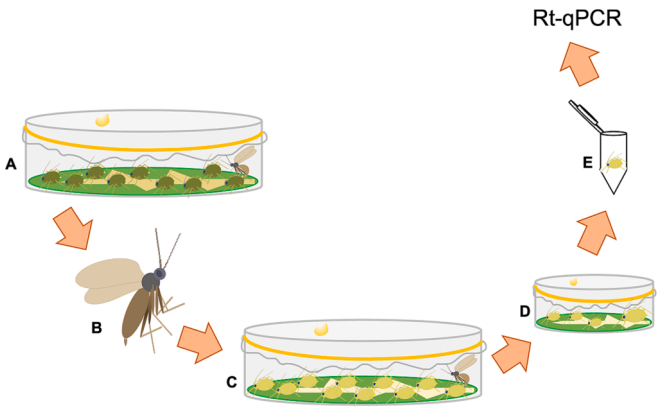

Fig. 1. Experimental design for testing horizontal transmission of *R. viridis* in *M. persicae* via *D. rapae*. A: Female *D. rapae* were introduced to bok choy discs in petri dishes with approximately 10 adult R + *M. persicae* and allowed to probe the aphids. B: Female *D. rapae* were removed. C: Female *D. rapae* were placed in petri dishes with bok choy with 10 uninfected *M. persicae* nymphs and allowed to probe aphids for 24 h. D: Female *D. rapae* were removed, and live aphids were placed individually on bok choy in petri dishes and kept until 3 nymphs were produced. E: Individual aphid adults were screened for *R. viridis* using RT-qPCR.

photoperiod of 16L:8D and 50–80% humidity for at least 7 generations before experiments commenced. On arrival, we separated mummies into groups of 50 and placed them in glass vials (95 mm diameter) covered with a fine mesh and a drop of organic honey as a food resource for emerged wasps. Emerged *D. rapae* were transferred to insect rearing cages (30 × 30 × 62 cm) with 6 bok choy plants and *M. persicae*.

For experiments, emerged wasps were left to mate for at least 24 h before being anesthetized with CO_2. Wasps were sexed under a dissecting microscope using a fine paint brush based on the presence of an ovipositor in females and differences in the number of antennal segments (13–15 in females and 16–17 in males) (Gazmer et al., 2015). The separated females were given a recovery time of 24 h after exposure to CO_2, and they were then transferred to the experimental units using a mouth aspirator.

2.4. Effects of nymphal instar on R. viridis density

To test whether the density of *R. viridis* changed as *M. persicae* nymphs developed (which may affect potential parasitism by *D. rapae*), we compared 3–10 individuals from each of the nymph instars of *M. persicae* for their *R. viridis* density using RT-qPCR. A general linear model (GLM) was run to test for differences among nymphal instars in density of *R. viridis*. This statistical test and most others used in this paper were performed in Minitab software (version. 19.2020.2.0 for Mac, Minitab, LLC).

2.5. Effects of R. viridis on parasitism by D. rapae against M. persicae at two densities

To determine how *R. viridis* affects parasitism by *D. rapae* and the fecundity of *M. persicae* in the presence of *D. rapae*, we placed synchronized fourth instar nymphs of R+ or R- (uninfected by *Rickettsiella*) *M. persicae* at a density of 5 or 20 nymphs on 3 bok choy leaf discs (35 mm) in a petri dish (100 × 20 mm) and exposed them to a single female *D. rapae* for 48 h at 18 °C. We initially trialed *D. rapae* against 2nd instar (3 days old) *M. persicae* nymphs, in which we found low parasitism rates and no significant effects of the infection with *R. viridis*. Petri dishes were covered with fine mesh to prevent parasitoid escape and one drop of organic honey was smeared on top of the mesh as a source of food. We performed this experiment with 30 replicates for each density and each of the R + and R- lines. We also included 10 control replicates without *D. rapae* for each combination of line and density. After 48 h, *D. rapae*

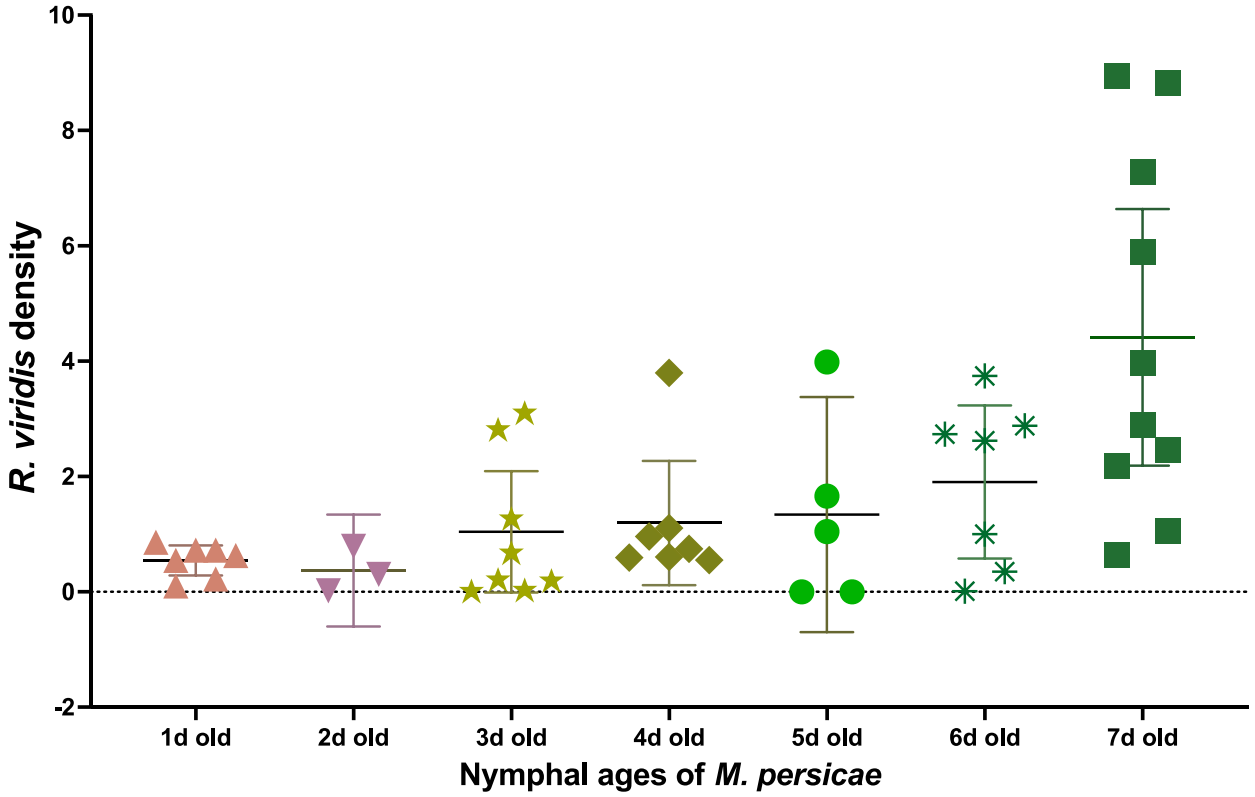

Fig. 2. Density of *R. viridis* at different ages of *M. persicae*. 10 nymph per time point was screened using RT- qPCR. Symbols denote the transformed (2^n) density of *R. viridis* in individual R + *M. persicae*. Lines and error bars show means with 95% confidence intervals.

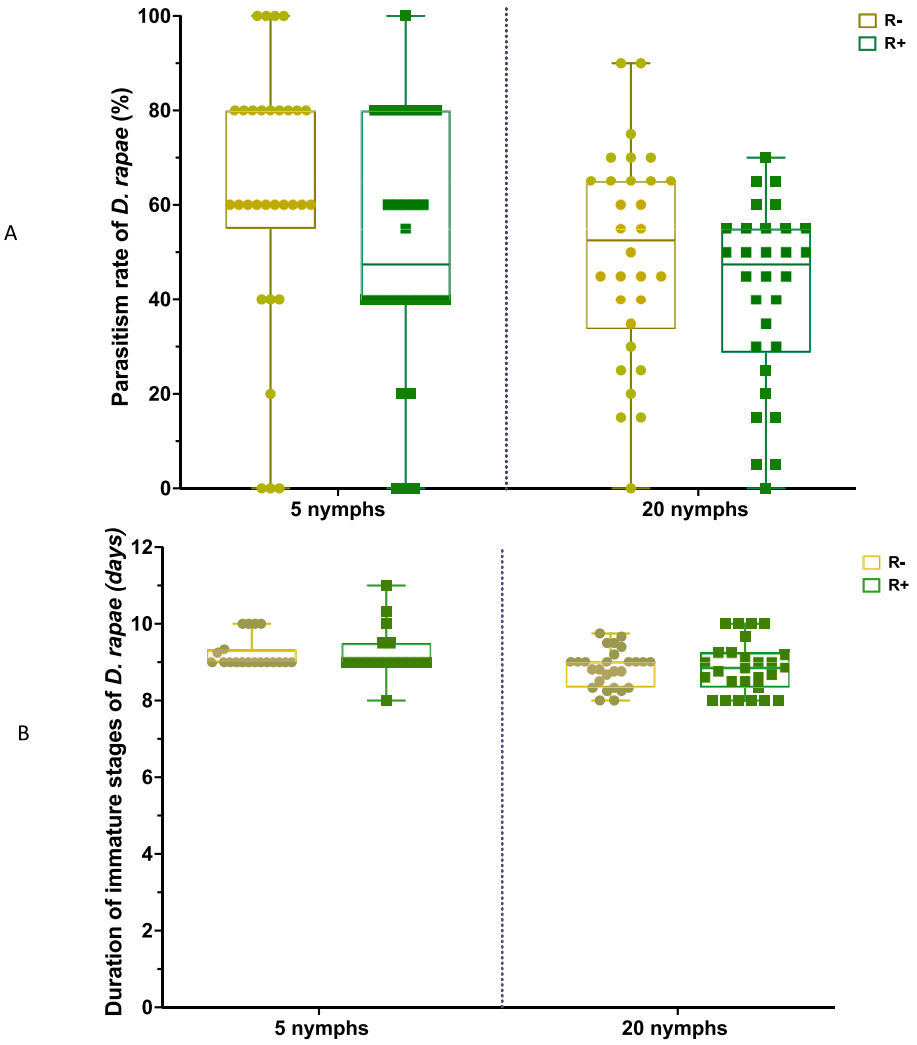

Fig. 3. Parasitism rate (%) (A), duration of immature stages (days) (B) of *D. rapae* exposed to R- or R+ *M. persicae* at densities of 5 or 20 fourth instar nymphs, emergence rate (%) and sex ratio (% female) of *D. rapae* against 5 (C) and 20 fourth instar nymph *M. persicae* (D). Each data point shows a single replicate which consisted of 20 aphids (R+/R-) in petri dishes (60 mm) and one female *D. rapae* at 18 °C, with boxes and whiskers for min to max range data except for C where binomial confidence intervals are presented.

were removed from petri dishes and live nymphs were transferred to new 60 mm petri dishes with 1 bok choy disc and maintained at 18 °C. The numbers of live aphids, mummies and emerged wasps were recorded daily. Mummies were collected in small vials covered with a fine mesh and wasps were stored in 100% ethanol.

For the 20 nymph treatment, we determined the parasitism rate of *D. rapae* by calculating the percentage of mummified aphids out of the initial aphid number in each replicate. Because data from this experiment was mostly not normally distributed even after transformation, we used Mann-Whitney U tests to compare parasitism rates among infected and uninfected aphids separately for each nymph density. Emergence rates were calculated by taking the percentage of mummies that emerged as wasps in each replicate and comparing them with Mann-Whitney U tests. We used the same approach to compare sex ratio (the percentage of emerging *D. rapae* that were female) and the duration of immature stages (eggs, larval stages, and pupae, averaged across replicate). Since quite a low number of emergences occurred in replicates with 5 nymphs, data across all replicates were combined in order to assess infection effects on emergence rate and sex ratio. Chi-square tests were undertaken on two-way contingency tables and emergence/sex ratios with binomial confidence intervals were computed using a Wilson test (Epitool online calculator: https://epitools.ausvet.com.au/cipropo rtion).

To test changes in fecundity of *M. persicae* due to *R. viridis* infection and the presence or absence of *D. rapae*, the daily count of the number of nymphs produced per replicate was recorded until all the aphids either transformed into mummies or died. The cumulative number of nymphs per aphid per day was assessed. A general linear model including the factors infection with *R. viridis* and the presence of *D. rapae* as well as aphid density (5 or 20 nymphs) was used to examine the effects of these variables on this fecundity measure after log transformation. We also considered the overall association between parasitism and fecundity when 20-nymphs were present by fitting a linear regression between parasitism rate and fecundity for infected and uninfected aphids. We examined whether the slope and intercept of the regression lines differed between the infected and uninfected aphids.

2.6. Effects of *R. viridis* on *D. rapae* preference

To test if infection with *R. viridis* affects the probing preferences of *D. rapae* females in *M. persicae*, we performed an experiment where individual *D. rapae* were provided a choice between R+ and R- *M. persicae*. Single mated female *D. rapae* were separated using CO_2 and kept in a CT cabinet at 18 °C for 24 h to ensure recovery. We exposed each female to

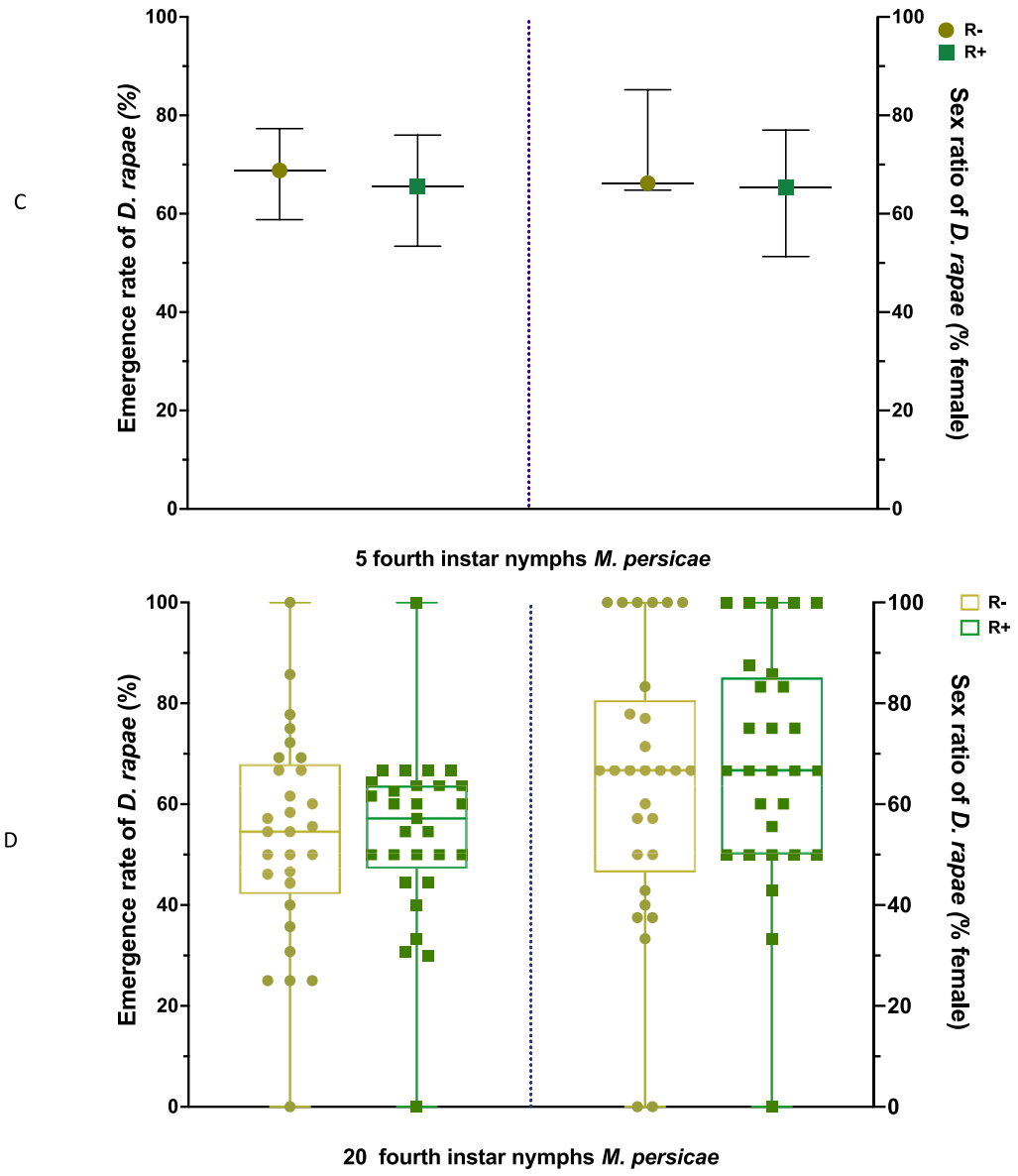

Fig. 3. (*continued*).

5 R+ and 5 R- nymphs of *M. persicae* (4th instar) inside petri dishes (30 × 6 mm) at room temperature and recorded their behavior with a compact camera (Casio EX-ZR1000) for 20 min. For each petri dish, we recorded the number of probing attempts against R+/R- *M. persicae* in the time intervals of 5 min. We distinguished between R+ and R- *M. persicae* based on color differences (dark green for R+ and light green for R-) (Gu et al., 2023). This experiment was performed with 13 replicates. A Wilcoxon Sign-Rank test was used to compare preference of *D. rapae* for *M. persicae* either infected or uninfected with *R. viridis* based on the relative number of probing attempts of R+ or R- aphids in each replicate.

2.7. Effects of D. rapae on the spread of R. viridis through M. persicae populations

To investigate the spread of *R. viridis* in *M. persicae* in the presence or absence of *D. rapae*, we placed a mixture of 50 R- and 50 R+ (frequency of 1:1) on one bok choy plant inside an insect rearing cage (30 × 30 × 62 cm, mesh 160 μm aperture to prevent escaping of aphids and parasitoids) at 18 °C. To ensure that aphids settled on plants before being exposed to parasitoids, 3 pairs of *D. rapae* were added to each cage after 24 h. Plants were replaced at 2-week intervals and 100 *M. persicae* from the previous plant were transferred to the new plant. The remaining aphids were stored in ethanol for subsequent analysis. We performed this experiment with 5 experimental replicates as described above and 5 controls where no *D. rapae* were added to insect cages and the initial aphid population was 30 R- and 30 R+ *M. persicae*. After 3 and 9 weeks, we estimated *R. viridis* infection frequencies by screening 12 aphids from each time point and replicate plant for *R. viridis* according to the procedures described above.

2.8. Horizontal and subsequent vertical transmission of R. viridis in M. persicae via D. rapae

We hypothesized that *D. rapae* is a potential candidate to facilitate horizontal transmission of *R. viridis* between different clones of *M. persicae* (multilocus genotypes Kyabram 98 and Elliotte 158) infesting canola in Victoria and designed an experiment to test this (Fig. 1). Female *D. rapae* were separated using CO_2 and kept in a CT cabinet at 18 °C for 24 h to recover. Afterwards, 15 female *D. rapae* were placed individually in 30 mm petri dishes containing 5 adult R+ *M. persicae*. Female *D. rapae* were observed until they probed the aphids at least 3

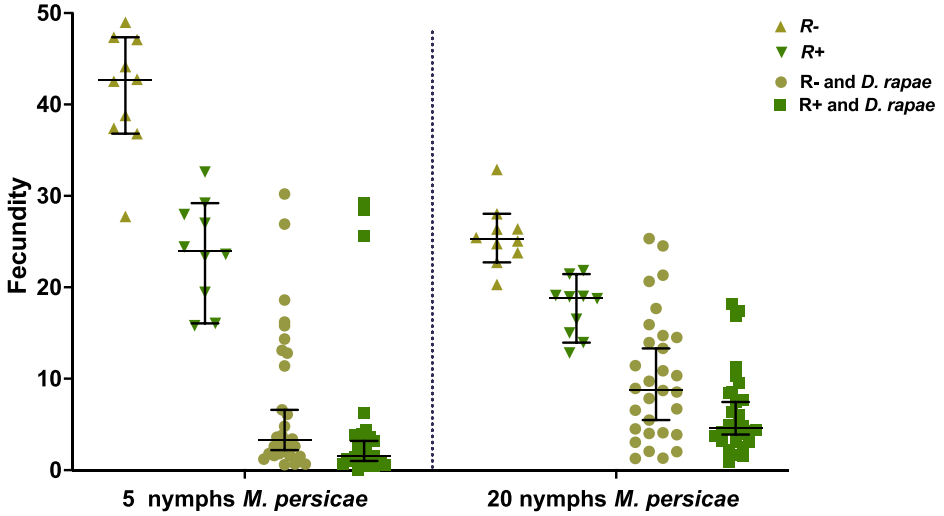

Fig. 4. Effects of infection with *R. viridis* on *M. persicae* fecundity per aphid at two densities of 5 or 20 fourth instar nymphs and in the presence or absence of *D. rapae*. Each data point shows a single replicate consisting of 5 aphids or 20 aphids (R+/R-) in petri dishes (60 mm) and one female *D. rapae* at 18 °C, with lines and error bars showing means and 95% confidence intervals.

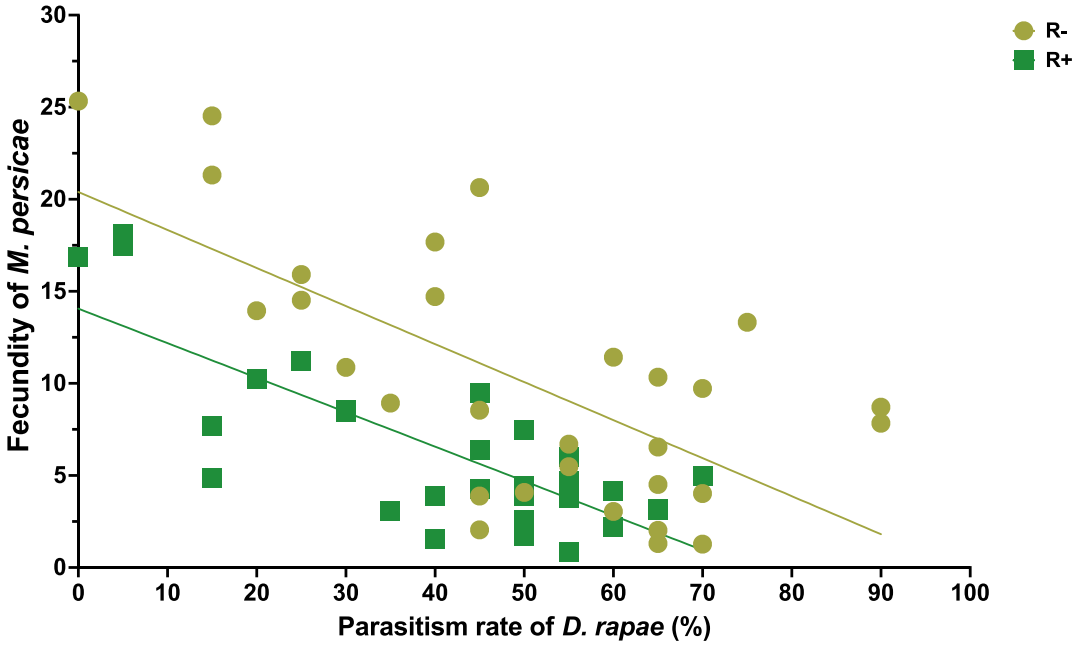

Fig. 5. Association between parasitism rate (%) and fecundity of R + and R- *M. persicae* at a density of 20 fourth instar nymphs. Lines indicate linear regression lines for R + and R- data.

times, then aspirated into 100 mm petri dishes containing 10 R- *M. persicae* on 3 bok choy leaf discs (35 mm). Female *D. rapae* were allowed to probe R- *M. persicae* for 48 h before being removed from petri dishes. Once *D. rapae* were removed, live aphids were placed in 30 mm petri dishes on bok choy leaf disc (25 mm) individually and maintained at 18 °C. Once mummies were formed, all live aphids were screened for *R. viridis* infection using RT-qPCR (see above).

The offspring of R- *M. persicae* samples which tested positive for *R. viridis* were transferred individually to new leaf discs in 30 mm petri dishes to evaluate vertical transmission of *R. viridis* in the following generations. Nymphs were reared to adulthood to produce at least 3 nymphs before screening their genomic DNA for *R. viridis*. This process was repeated to monitor the vertical transmission of *R. viridis* across generations.

3. Results

3.1. Effects of nymphal instar on R. viridis density

R. viridis density differed among instars (GLM: $F_{6,40} = 4.79$, $P = 0.001$) with a gradual increase over developmental stages of *M. persicae* nymphs (Fig. 2) in which 1-day-old nymphs represent first instars, 3-day-old nymphs represent second instars, 5-day-old nymphs represent third instars, and 7-day-old nymphs represent fourth instars.

3.2. Effects of R. viridis on parasitism by D. rapae against M.persicae at two densities

We compared the parasitism rate of R+ and R- *M. persicae* by *D. rapae* at densities of 5 and 20 fourth instar nymphs. Mann-Whitney U tests

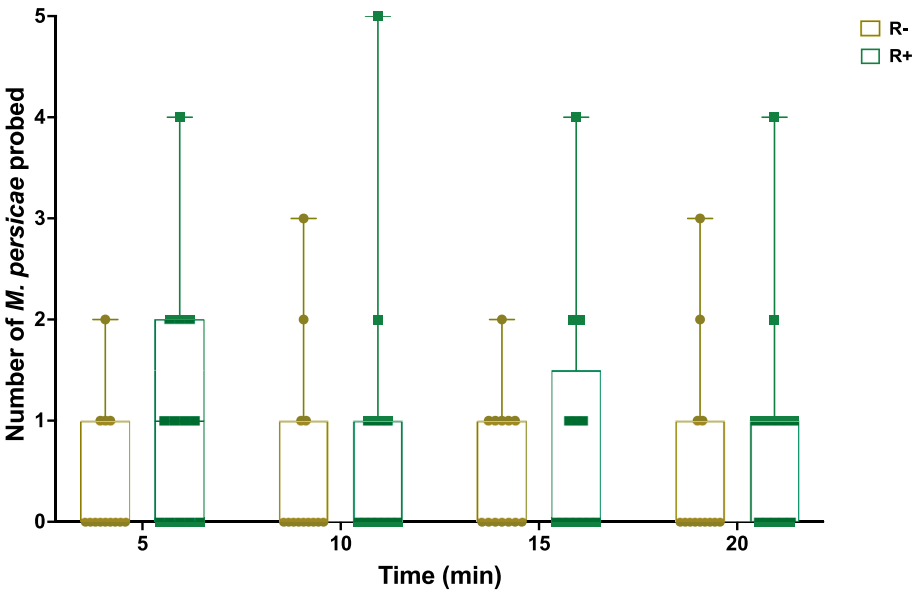

Fig 6. Effects of *R. viridis* on the number of probing attempts of *D. rapae* against R+/R- *M. persicae* in time intervals of 5 min. Each data point shows a single replicate which consisted of 5 R+ and 5 R- aphids in petri dishes (30 mm) and one female *D. rapae* at 18 °C, with boxes and whiskers for min to max range data.

showed that infection with *R. viridis* had no significant effect on the parasitism rate of *D. rapae* (5-nymphs: Z = 1.63, $P = 0.072$, 20-nymphs: Z = 1.52, $P = 0.128$, Fig. 3A). The duration of immature stages of *D. rapae* was also not significantly affected by *R. viridis* infection in *M. persicae* (5-nymphs: Z = -2.49, $P = 0.887$; 20-nymphs: Z = 0.10, $P = 0.917$, Fig. 3B). Moreover, for the 5-nymph treatment, the emergence rate ($X^2 = 1.127$, df = 1, $P = 0.288$, Fig. 3C) and sex ratio ($X^2 = 0.097$, df = 1, $P = 0.755$, Fig. 3C) of *D. rapae* showed no significant difference due to the presence of *R. viridis*, where we only presented pooled data and binomial confidence intervals due to low sample sizes per replicate (Fig. 3C). These patterns are consistent with the 20-nymph treatment, in which the emergence rate (Z = -0.06, $P = 0.926$, Fig. 3D) and sex ratio (Z = -0.54, $P = 0.172$, Fig. 3D) of *D. rapae* were also similar between the R+ and R- treatments.

3.3. Fecundity of M. persicae

We tested whether the presence of *D. rapae* interacts with the infection to affect fitness costs of *R. viridis* by comparing the fecundity of R+ and R- *M. persicae* in the presence or absence of parasitoids at two nymphal densities (Fig. 4). A general linear model on log transformed data showed that there was a significant effect (GLM: $F_{1,147} = 12.6$, $P = 0.001$) of infection with *R. viridis* on fecundity of *M. persicae*, regardless of aphid density (GLM: $F_{1,147} = 0.46$, $P = 0.50$). There was also a substantial effect of the presence of *D. rapae* (GLM: $F_{1,147} = 30.17$, $P < 0.01$), where treatments with wasps had greatly reduced fecundity. Moreover, a strong interaction between *D. rapae* presence and aphid density was found (GLM: $F_{1,147} = 18.24$, $P < 0.01$) with a lower decrease in fecundity due to *D. rapae* when aphid density was higher (i. e. 20-nymphs), compared to the 5-nymph treatment (Fig. 4).

3.4. Association between parasitism rate (%) and fecundity of M. persicae

A negative relationship between parasitism rate and fecundity of 20 fourth instar nymphs was found for both the R + and R- aphids (Fig. 5). Linear regression was used to test if parasitism rate and infection with *R. viridis* significantly predicted fecundity of *M. persicae*. Overall, parasitism had a significant effect on fecundity ($F_{1,\ 147} = 24.75$, $P < 0.001$) and infection did as well ($F_{1,\ 147} = 12.60$, $P = 0.001$) but the interaction between parasitism and infection was not significant ($F_{1,\ 147} = 0.46$, $P = 0.50$). The intercept and slope (with standard errors) of the regression line for R- was y = 20.40 (1.19) − 0.206 (0.035) and for R+ it was y = 14.06 (2.66) − 0.187 (0.035). These patterns indicate similar regression slopes for the R+ and R- aphids but with a lower intercept for the R+ aphids with population collapse due to parasitism (fecundity = 0) predicted to occur earlier when aphids are infected.

3.5. Effects of R. viridis on D. rapae preference

To test whether *R. viridis* infection influenced the probing behavior of *D. rapae*, we recorded probing attempts of individual female *D. rapae* against mixed groups of 5 R- and 5 R+ *M. persicae* (4th instar nymphs) which differed in body color for a period of 20 min. A Wilcoxon Sign-Rank test suggests a clear preference towards *M. persicae* infected with *R. viridis*, in terms of the relative number of probing attempts (0–5) of R + or R- *M. persicae* within the replicates during the first 20 min of their interactions (Z = -2.285, $P = 0.022$), with infected aphids being preferred in 11 of 13 replicates. There were also more R+ aphids probed on average compared to R- aphids at each 5 min interval (Fig. 6).

3.6. Effects of D. rapae on the spread of M. persicae infected with R. viridis

Our results indicated an initial persistence of the *R. viridis* infection in the *M. persicae* populations 3 weeks after exposure to *D. rapae* at 18 °C (Fig. 7A). However, the infection reduced at week 9 in two of the three replicates where the population was not completely suppressed by *D. rapae* but increased in the other replicate, although the confidence intervals overlapped (Fig. 6A). Note that in two replicates (2 and 4) parasitism was 100% after week 3. In the absence of *D. rapae*, *R. viridis* spread in the first three weeks in all replicates, followed by a consistent decrease in the next 6 weeks (Fig. 7B).

3.7. Horizontal and subsequent vertical transmission of R. viridis in M. persicae via D. rapae

We tested the potential for *D. rapae* to facilitate horizontal transmission of *R. viridis* between *M. persicae* individuals. In two separate experiments with different genotypes as recipients, we found horizontal transmission rates of 10% (4/40 testing positive for *R. viridis*) and 6% (5/83 positive) for multilocus genotypes Kyabram 98 and Elliotte 158

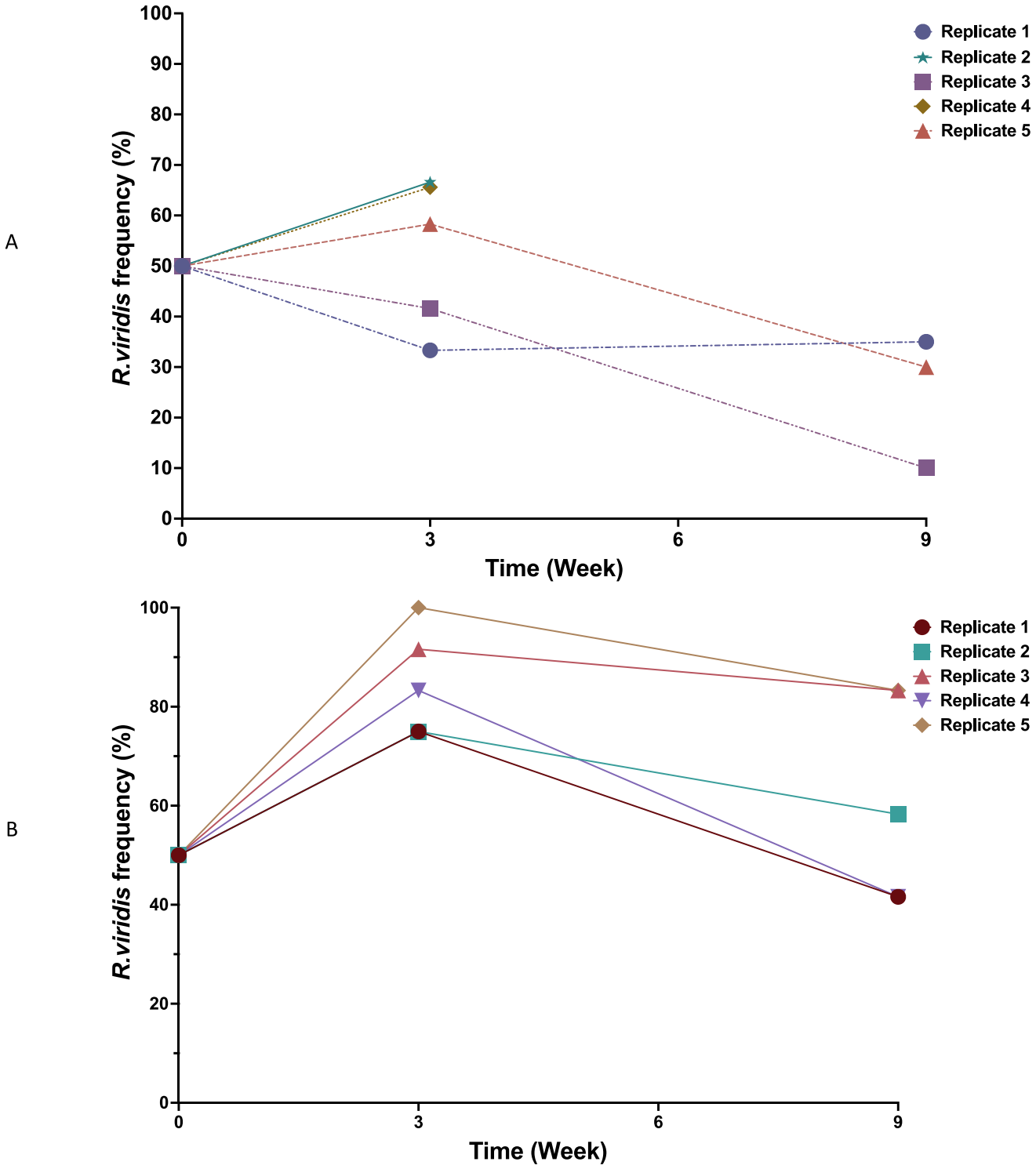

Fig. 7. Changes in frequency of *R. viridis* infection among *M. persicae* populations (%) in the presence of *D. rapae* (A) and absence of *D. rapae* (B) at 18 °C. Data points for A and B show the frequency of *M. persicae* R + using RT- qPCR in which the initial number of aphids placed on each bok choy plant and exposed to *D. rapae* were 50:50 *M. persicae* R-/ R+ (ratio of 1:1) and 30:30 for control treatments without *D. rapae* (12 aphids were tested per time point, per replicate cage). All *M. persicae* in replicates 1 and 2 were either mummified or dead after 3 weeks meaning that no aphids were left to continue experiment (A).

respectively. Our results suggest that *D. rapae* could potentially facilitate horizontal transmission of *R. viridis* among different clones of *M. persicae*.

Subsequently, *R. viridis* was transmitted from mother to offspring over two generations before the infection was lost in multilous genotype Kyabram 98, whereas in Elliotte 158 it persisted through one generation. The *R. viridis* infection rate in the first generation was 33.3% (5/15 positive) for Kyabram 98 and 20% (2/10 positive) for Elliotte 158, followed by a decline in both infection rate and density in Kyabram 98 (20% infection rate, 5/25 positive) and a loss (0/10 positive) in Elliotte 158 (Fig. 8). The endosymbiont may therefore be transmitted through the parasitoid and then in some cases even persist across generations.

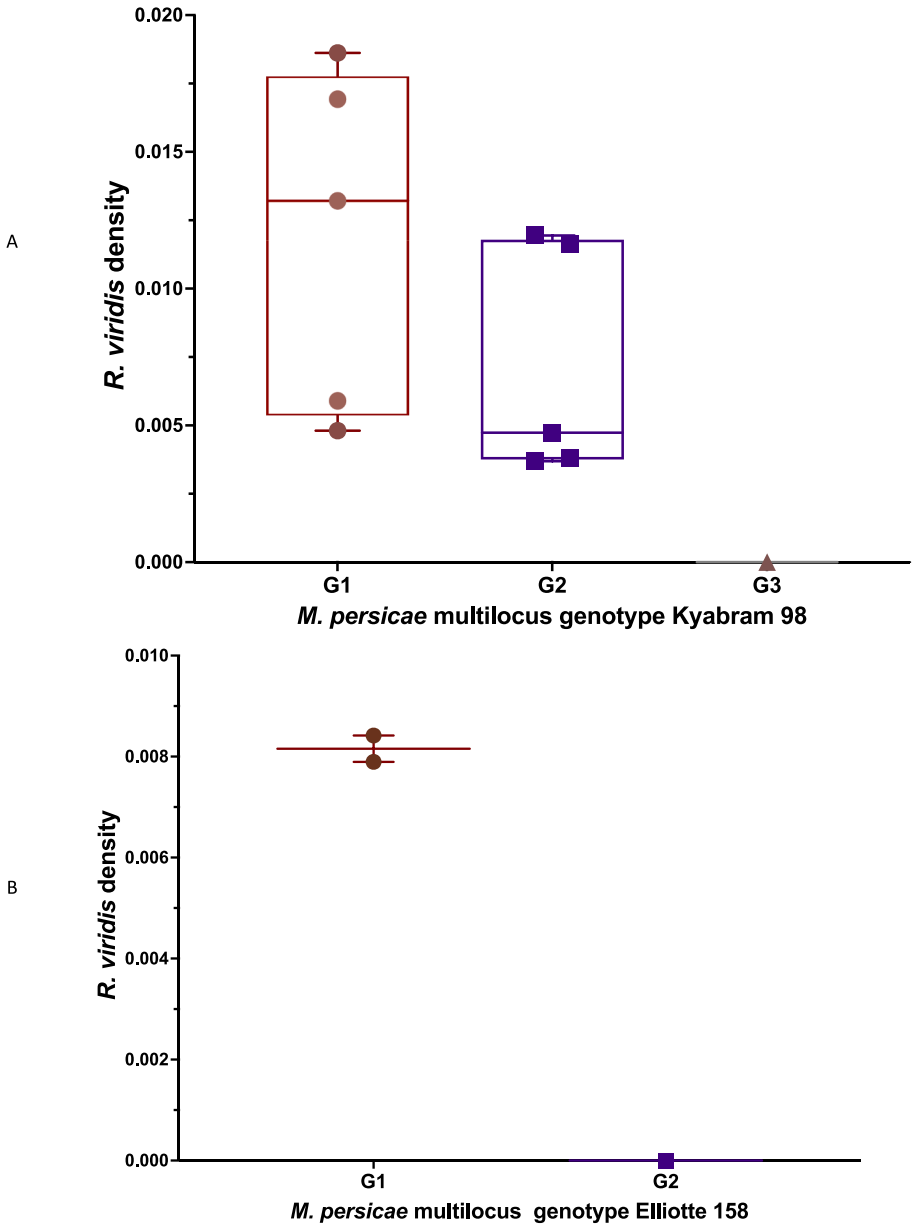

Fig. 8. Box plots of density of *R. viridis* transmitted vertically from mother to offspring in two different clones of *M. persicae* after being transmitted horizontally via *D. rapae*. Changes in density of *R. viridis* were tracked across three generations of *M. persicae* multilocus genotype 98 (A) and two generations of multilocus genotype 158 (B) using RT-qPCR. Symbols denote the transformed (2^n) density of *R. viridis* in individual *M. persicae* R + with boxes and whiskers for min to max range data.

4. Discussion

Our results indicate that *R. viridis* has no detectable effect on susceptibility to the specialist parasitoid *D. rapae* in *M. persicae* populations, despite a parasitoid preference for probing infected aphids. We also found a low level of horizontal and subsequent vertical transmission mediated by this parasitoid. Our data also confirmed persistence of the *R. viridis* infection in *M. persicae* populations regardless of the presence of parasitoids, despite strong costs to host fecundity at 18 °C. As discussed elsewhere (Gu et al., 2023), the rapid spread of this facultative endosymbiont in host populations seems to be mediated by horizontal transmission via leaf tissue which may allow it to spread in clonal species.

Facultative endosymbionts such as *H. defensa* (Oliver et al., 2005, 2003), *R. insecticola* (Vorburger et al., 2013), *Serratia symbiotica* (Guo et al., 2017; Russell and Moran, 2005) and *Fukatsuia symbiotica* (Heyworth and Ferrari, 2015) protect their hosts against parasitoids, within the context of laboratory assays. However, we found no clear evidence of a protective effect of *R. viridis* against *D. rapae* among *M. persicae*. This result was established with fourth instar nymphs in which we found the highest titer of *R. viridis* (Fig. 2) and parasitism. We also found no effect in pilot experiments on parasitism of second instar nymphs thought to be preferred by *D. rapae* (Soni and Kumar, 2021) but for which we obtained lower parasitism (Fig. S1).

Cornicle secretions of *A. pisum,* together with sesquiterpene *trans*-b-farnesene (EBF: the main component of aphid alarm pheromone secreted from the cornicles), have been described as a contact kairomone for *Aphidius ervi (*Russell et al., 2003*)* and the quantity of this volatile compound seems to play a role for discriminating between infected and uninfected hosts (Oliver et al., 2012). Since the density of aphids seems to be directly related to the secretion of alarm pheromone, we compared two densities of nymphs (5 and 20). The 20-nymph treatment showed a lower parasitism rate (Fig. 3) although this would have translated into a higher rate of parasitism per parasitoid present.

Regardless, at both densities there were no clear effects of *R. viridis* on parasitism by *D. rapae* even though density effects were clearly having an impact on parasitism rate which can determine potential functions of alarm pheromone as a contact kairomone for *D. rapae*. Furthermore, our results suggest that *R. viridis* presence does not affect development time, sex ratio or the emergence rate of *D. rapae*.

Rickettsiella viridis affects aphid body color which is considered as an important ecological and evolutionary trait (Gu et al., 2023; Tsuchida et al., 2014). In *A. pisum*, greenish body color is caused by polycyclic quinone pigments (Tsuchida et al., 2010). Previous studies demonstrated that the parasitoid *A. ervi* prefers to oviposit in green morphs of *A. pisum* compared to red morphs (Bilodeau et al., 2013; Libbrecht et al., 2007; Losey et al., 1997). While the nature of the color change here is different, *Rickettsiella viridis* may have a similar effect on *M. persicae* as our data indicated a probing bias towards infected *M. persiace*. However, this effect did not translate into a detectably higher rate of parasitism. It is possible that *R. viridis* does provide some protection against parasitism that is offset by an increased probing rate, but confirming this hypothesis and determining the basis of differences in probing behavior requires further study. In pea aphids, lambda bacteriophage inside the genome of endosymbionts such as *H. defensa* could be involved in lowering parasitism rates despite a high probing incidence; this lambda bacteriophage encodes eukaryotic toxins that prevent parasitoid larvae and eggs from further development inside their hosts (Moran et al., 2005; Oliver et al., 2009). However, lambda phages have not been identified in the genome of *R. viridis* although its genome does carry a prophage region (Nikoh et al., 2018).

Our data indicate that *M. persicae* individuals harboring *R. viridis* experienced a significant decline in their lifetime fecundity in the presence of parasitoids, consistent with data in Gu et al. (2023). Similar costs were reported in aphids infected with *Fukatsuia symbiotica* endosymbionts under benign conditions (Heyworth and Ferrari, 2015) and most *H. defensa* strains (Simon et al., 2011; Tsuchida et al., 2014; Vorburger et al., 2013; Vorburger and Gouskov, 2011). Facultative endosymbionts usually have stronger fitness costs in artificially infected hosts compared to natural infections which could be associated with incompatibility between aphid and host genotypes (Heyworth and Ferrari, 2015).

We found that *D. rapae* may facilitate horizontal transmission of *R. viridis* in *M. persicae* clones which aligns with previous studies confirming horizontal transmission of some endosymbionts via parasitoids and oral acquisitions (Darby and Douglas, 2003; Gehrer and Vorburger, 2012; Heath et al., 1999; Kellner, 2002; Schilthuizen and Stouthamer, 1997). However, transmission via parasitoid ovipositor did not result in stable establishment of *R. viridis* in two different clones where the infection was lost in early generations. This could be associated with lower densities of *R. viridis* transferred via parasitoids when compared to microinjection (Gu et al., 2023). The transmission success of *R. viridis* may have differed between the two clones. Although the reasons for this difference are unclear, it has previously been noted that horizontal transmission of another symbiont (*H. defensa*) can vary among aphid clones which is only partly related to symbiont density (Kaech and Vorburger, 2021). Weak protection against parasitoids and potential effects on horizontal transmission may contribute to variation in the persistence of the infection across generations. Nevertheless, occasional parasitoid-mediated transmission may contribute to the transmission of this endosymbiont across aphid species which occupy different host plants but are attacked by the same parasitoid species.

Our study indicates that there may be potential benefits of simultaneously releasing *R. viridis* and *D. rapae* in crops early in the aphid season when *M. persicae* populations are expanding. The *R. viridis* infection not only reduces fecundity but can also spread through *M. persicae* populations. Furthermore, the parasitoids provide an additional level of control and may facilitate some additional horizontal transmission of *R. viridis* among *M. persicae* populations. With the overall population of *M. persicae* reduced due to the presence of *R. viridis* and parasitoids, it may be possible to maintain aphid populations under an economic threshold or even cause a local collapse as happened in some of our laboratory populations (Fig. 6). Such integrated pest management strategies may help reduce reliance on pesticides and help increase the impact of biocontrol agents.

CRediT authorship contribution statement

Safieh Soleimannejad: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. **Perran A. Ross:** Conceptualization, Methodology, Supervision, Supervision. **Ary A. Hoffmann:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2023.105377.

References

Asplen, M.K., Bano, N., Brady, C.M., Desneux, N., Hopper, K.R., Malouines, C., Oliver, K. M., White, J.A., Heimpel, G.E., 2014. Specialisation of bacterial endosymbionts that protect aphids from parasitoids: Defensive symbiosis in the cowpea aphid. Ecol. Entomol. 39, 736–739. https://doi.org/10.1111/een.12153.

Bass, C., Puinean, A.M., Zimmer, C.T., Denholm, I., Field, L.M., Foster, S.P., Gutbrod, O., Nauen, R., Slater, R., Williamson, M.S., 2014. The evolution of insecticide resistance in the peach potato aphid, *Myzus persicae*. Insect Biochem. Mol. Biol. 51, 41–51. https://doi.org/10.1016/j.ibmb.2014.05.003.

Baumann, P., 2005. Biology bacteriocyte-associated endosymbionts of plant sap-sucking insects. Annu. Rev. Microbiol. 59, 155–189. https://doi.org/10.1146/annurev.micro.59.030804.121041.

Bilodeau, E., Guay, J.-F., Turgeon, J., Cloutier, C., 2013. Survival to parasitoids in an insect hosting defensive symbionts: a multivariate approach to polymorphic traits affecting host use by its natural enemy. PLoS One 8, e60708.

Blackman, R.L., Eastop, V.F., 2000. Aphids on the world's crops: an identification and information guide, 2nd ed. Wiley, Chichester Weinheim.

Blande, J.D., Pickett, J.A., Poppy, G.M., 2004. Attack rate and success of the parasitoid *Diaeretiella rapae* on specialist and generalist feeding aphids. J. Chem. Ecol. 30, 1781–1795. https://doi.org/10.1023/B:JOEC.0000042401.52088.54.

Brandt, J.W., Chevignon, G., Oliver, K.M., Strand, M.R., 2017. Culture of an aphid heritable symbiont demonstrates its direct role in defence against parasitoids. Proc. Biol. Sci. 20171925 https://doi.org/10.1098/rspb.2017.1925.

Chirgwin, E., Yang, Q., Umina, P.A., Gill, A., Soleimannejad, S., Gu, X., Ross, P., Hoffmann, A.A., 2022. Fungicides have transgenerational effects on *Rhopalosiphum padi* but not their endosymbionts. Pest Man Sci 78, 4709–4718. https://doi.org/10.1002/ps.7091.

Cole, R.A., 1997. Comparison of feeding behaviour of two *Brassica* pests *Brevicoryne brassicae* and *Myzus persicae* on wild and cultivated brassica species. Ent Exp Appl 85, 135–143. https://doi.org/10.1046/j.1570-7458.1997.00243.x.

Congdon, B.S., Baulch, J.R., Coutts, B.A., 2020. Impact of Turnip yellows virus infection on seed yield of an open-pollinated and hybrid canola cultivar when inoculated at different growth stages. Virus Res. 277, 197847 https://doi.org/10.1016/j.virusres.2019.197847.

Darby, A.C., Douglas, A.E., 2003. Elucidation of the transmission patterns of an insect-borne bacterium. Appl. Environ. Microbiol. 69, 4403–4407. https://doi.org/10.1128/AEM.69.8.4403-4407.2003.

De Little, S.C., Edwards, O., Van Rooyen, A.R., Weeks, A., Umina, P.A., 2017. Discovery of metabolic resistance to neonicotinoids in green peach aphids (*Myzus persicae*) in Australia. Pest Manag. Sci. 73, 1611–1617. https://doi.org/10.1002/ps.4495.

DeBach, P., Rosen, D., 1991. Biological Control by Natural Enemies, 2nd. ed. Cambridge Univ. Press, Cambridge.

Desneux, N., Wajnberg, E., Fauvergue, X., Privet, S., Kaiser, L., 2004. Oviposition behaviour and patch-time allocation in two aphid parasitoids exposed to deltamethrin residues. Ent Exp Appl 112, 227–235. https://doi.org/10.1111/j.0013-8703.2004.00198.x.

Douglas, A.E., 1989. Mycetocyte symbiosis in insects. Biological Reviews of the Cambridge Philosophical Society 64, 409–434. https://doi.org/10.1111/j.1469-185x.1989.tb00682.x.

Elliott, N.C., Backoulou, G.F., Giles, K.L., Royer, 2014. Aphids and parasitoids in wheat and nearby canola fields in central Oklahoma. Southwest Entomol 39, 23–28. https://doi.org/10.3958/059.039.0103.

Gazmer, R., Gupta, M.K., Singh, M.D., 2015. Biology of *Diaeretiella rapae* (Mc Intosh) (Hymenoptera: Aphidiidae) on Cabbage Aphid (*Brevicoryne brassicae* Linnaeus) and influence of host age on the developmental duration. J Biol Cont 29, 38. https://doi.org/10.18641/jbc/29/1/75802.

Gehrer, L., Vorburger, C., 2012. Parasitoids as vectors of facultative bacterial endosymbionts in aphids. Biol. Lett. 8, 613–615. https://doi.org/10.1098/rsbl.2012.0144.

Gu, X., Ross, P.A., Gill, A., Yang, Q., Ansermin, E., Sharma, S., Soleimannejad, S., Sharma, K., Callahan, A., Brown, C., Umina, P.A., Kristensen, T.N., Hoffmann, A.A., 2023. A rapidly spreading deleterious aphid endosymbiont that uses horizontal as well as vertical transmission. PNAS 120. https://doi.org/10.1073/pnas.2217278120.

Gullan, P.J., Cranston, P.S., 2014. The Insects: an Outline of Entomology, 5th ed. Wiley Blackwell, Chichester.

Guo, J., Hatt, S., He, K., Chen, J., Francis, F., Wang, Z., 2017. Nine facultative endosymbionts in aphids. A review. J Asia-Pacific Entomol 20, 794–801. https://doi.org/10.1016/j.aspen.2017.03.025.

Hardin, M.R., Benrey, B., Coll, M., Lamp, W.O., Roderick, G.K., Barbosa, P., 1995. Arthropod pest resurgence: an overview of potential mechanisms. Crop Prot. 14, 3–18. https://doi.org/10.1016/0261-2194(95)91106-P.

Heath, B.D., Butcher, R.D., Whitfield, W.G., Hubbard, S.F., 1999. Horizontal transfer of *Wolbachia* between phylogenetically distant insect species by a naturally occurring mechanism. Curr. Biol. 9, 313–316. https://doi.org/10.1016/s0960-9822(99)80139-0.

Heyworth, E.R., Ferrari, J., 2015. A facultative endosymbiont in aphids can provide diverse ecological benefits. J. Evol. Biol. 28, 1753–1760. https://doi.org/10.1111/jeb.12705.

Kaech, H., Vorburger, C., 2021. Horizontal transmission of the heritable protective endosymbiont *Hamiltonella defensa* depends on titre and haplotype. Front. Microbiol. 11, 628755 https://doi.org/10.3389/fmicb.2020.628755.

Kellner, R.L.L., 2002. Interspecific transmission of *Paederus* endosymbionts: relationship to the genetic divergence among the bacteria associated with pederin biosynthesis. Chemoecology 12, 133–138. https://doi.org/10.1007/s00012-002-8338-1.

Khatri, D., He, X.Z., Wang, Q., 2017. Effective biological control depends on life history strategies of both parasitoid and its host: Evidence from *Aphidius colemani-Myzus persicae* system. J. Econ. Entomol. 110, 400–406. https://doi.org/10.1093/jee/tow324.

Libbrecht, R., Gwynn, D.M., Fellowes, M.D.E., 2007. *Aphidius ervi* preferentially attacks the green morph of the pea aphid, *Acyrthosiphon pisum*. J. Insect Behav. 20, 25–32. https://doi.org/10.1007/s10905-006-9055-y.

Losey, J.E., Harmon, J., Ballantyne, F., Brown, C., 1997. A polymorphism maintained by opposite patterns of parasitism and predation. Nature 388, 269–272. https://doi.org/10.1038/40849.

Łukasik, P., van Asch, M., Guo, H., Ferrari, J., Godfray, H.C.J., 2013. Unrelated facultative endosymbionts protect aphids against a fungal pathogen. Ecol. Lett. 16, 214–218. https://doi.org/10.1111/ele.12031.

Moran, N.A., Russell, J.A., Koga, R., Fukatsu, T., 2005. Evolutionary relationships of three new species of Enterobacteriaceae living as symbionts of aphids and other insects. Appl. Environ. Microbiol. 71, 3302–3310. https://doi.org/10.1128/AEM.71.6.3302-3310.2005.

Nikoh, N., Tsuchida, T., Maeda, T., Yamaguchi, K., Shigenobu, S., Koga, R., Fukatsu, T., 2018. Genomic insight into symbiosis-induced insect color change by a facultative bacterial endosymbiont, "*Candidatus Rickettsiella viridis*". MBio 9, e00890–e918. https://doi.org/10.1128/mBio.00890-18.

Oliver, K.M., Russell, J.A., Moran, N.A., Hunter, M.S., 2003. Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. PNAS 100, 1803–1807. https://doi.org/10.1073/pnas.0335320100.

Oliver, K.M., Moran, N.A., Hunter, M.S., 2005. Variation in resistance to parasitism in aphids is due to symbionts not host genotype. PNAS 102, 12795–12800. https://doi.org/10.1073/pnas.0506131102.

Oliver, K.M., Degnan, P.H., Hunter, M.S., Moran, N.A., 2009. Bacteriophages encode factors required for protection in a symbiotic mutualism. Science 325, 992–994. https://doi.org/10.1126/science.1174463.

Oliver, K.M., Degnan, P.H., Burke, G.R., Moran, N.A., 2010. Facultative symbionts in aphids and the horizontal transfer of ecologically important traits. Annu. Rev. Entomol. 55, 247–266. https://doi.org/10.1146/annurev-ento-112408-085305.

Oliver, K.M., Noge, K., Huang, E.M., Campos, J.M., Becerra, J.X., Hunter, M.S., 2012. Parasitic wasp responses to symbiont-based defense in aphids. BMC Biol. 10, 11. https://doi.org/10.1186/1741-7007-10-11.

Peccoud, J., Mahéo, F., de la Huerta, M., Laurence, C., Simon, J.-C., 2015. Genetic characterisation of new host-specialised biotypes and novel associations with bacterial symbionts in the pea aphid complex. Insect Conserv Divers 8, 484–492. https://doi.org/10.1111/icad.12131.

Pike, K.S., Starý, P., Miller, T., Allison, D., Graf, G., Boydston, L., Miller, R., Gillespie, R., 1999. Host range and habitats of the aphid parasitoid *Diaeretiella rapae* (Hymenoptera: Aphidiidae) in Washington State. Environ. Entomol. 28, 61–71. https://doi.org/10.1093/ee/28.1.61.

Polin, S., Le Gallic, J.-F., Simon, J.-C., Tsuchida, T., Outreman, Y., 2015. Conditional reduction of predation risk associated with a facultative symbiont in an insect. PLoS One 10, e0143728.

Russell, J.A., Latorre, A., Sabater-Muñoz, B., Moya, A., Moran, N.A., 2003. Side-stepping secondary symbionts: widespread horizontal transfer across and beyond the Aphidoidea. Mol. Ecol. 12, 1061–1075. https://doi.org/10.1046/j.1365-294x.2003.01780.x.

Russell, J.A., Moran, N.A., 2005. Horizontal transfer of bacterial symbionts: heritability and fitness effects in a novel aphid host. Appl. Environ. Microbiol. 71, 7987–7994. https://doi.org/10.1128/AEM.71.12.7987-7994.2005.

Schilthuizen, M., Stouthamer, R., 1997. Horizontal transmission of parthenogenesis-inducing microbes in *Trichogramma* wasps. Proc. Biol. Sci. 264, 361–366. https://doi.org/10.1098/rspb.1997.0052.

Schliephake, E., Graichen, K., Rabenstein, F., 2000. Investigations on the vector transmission of the Beet mild yellowing virus (BMYV) and the Turnip yellows virus (TuYV). J Plant Diseases Prot 81–87.

Shigenobu, S., Watanabe, H., Hattori, M., Sakaki, Y., Ishikawa, H., 2000. Genome sequence of the endocellular bacterial symbiont of aphids *Buchnera* sp. APS 407, 6.

Silva, R.J., Cividanes, F.J., Pedroso, E.C., Sala, S.R.D., 2011. Host quality of different aphid species for rearing *Diaeretiella rapae* (McIntosh) (Hymenoptera: Braconidae). Neotrop. Entomol. 40, 477–482.

Simon, J.-C., Boutin, S., Tsuchida, T., Koga, R., Le Gallic, J.-F., Frantz, A., Outreman, Y., Fukatsu, T., 2011. Facultative symbiont infections affect aphid reproduction. PLoS One 6, e21831.

Soni, S., Kumar, S., 2021. Efficacy of the parasitoid, *Diaeretiella rapae* (McIntosh) (Hymenoptera: Braconidae) against *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) infesting rapeseed-mustard. J Asia-Pacific Entomol 24, 912–917. https://doi.org/10.1016/j.aspen.2021.07.019.

Tsuchida, T., Koga, R., Horikawa, M., Tsunoda, T., Maoka, T., Matsumoto, S., Simon, J.-C., Fukatsu, T., 2010. Symbiotic bacterium modifies aphid body color. Science 330, 1102–1104. https://doi.org/10.1126/science.1195463.

Tsuchida, T., Koga, R., Fujiwara, A., Fukatsu, T., 2014. Phenotypic effect of "*Candidatus Rickettsiella viridis*", a facultative symbiont of the pea aphid (*Acyrthosiphon pisum*), and its interaction with a coexisting symbiont. Appl. Environ. Microbiol. 80, 525–533. https://doi.org/10.1128/AEM.03049-13.

Umina, P.A., Edwards, O., Carson, P., Van Rooyen, A., Anderson, A., 2014. High levels of resistance to carbamate and pyrethroid chemicals widespread in Australian *Myzus persicae* (Hemiptera: Aphididae) populations. J. Econ. Entomol. 107, 1626–1638. https://doi.org/10.1603/EC14063.

Umina, P.A., Bass, C., van Rooyen, A., Chirgwin, E., Arthur, A.L., Pym, A., Mackisack, J., Mathews, A., Kirkland, L., 2022. Spirotetramat resistance in *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) and its association with the presence of the A2666V mutation. Pest Manag. Sci. 78, 4822–4831. https://doi.org/10.1002/ps.7103.

Van Emden, H.F., Harrington, R., 2017. Aphids as Crop Pests, 2nd ed. CABI, Wallingford.

Vorburger, C., Ganesanandamoorthy, P., Kwiatkowski, M., 2013. Comparing constitutive and induced costs of symbiont-conferred resistance to parasitoids in aphids. Ecol. Evol. 3, 706–713. https://doi.org/10.1002/ece3.491.

Vorburger, C., Gouskov, A., 2011. Only helpful when required: a longevity cost of harbouring defensive symbionts. J. Evol. Biol. 24, 1611–1617. https://doi.org/10.1111/j.1420-9101.2011.02292.x.

Vorburger, C., Lancaster, M., Sunnucks, P., 2003. Environmentally related patterns of reproductive modes in the aphid *Myzus persicae* and the predominance of two "superclones" in Victoria, Australia. Mol. Ecol. 12, 3493–3504. https://doi.org/10.1046/j.1365-294x.2003.01998.x.

Ward, S.E., Umina, P.A., Macfadyen, S., Hoffmann, A.A., 2021. Hymenopteran parasitoids of aphid pests within Australian grain production landscapes. Insects 12, 44. https://doi.org/10.3390/insects12010044.

Yang, Q., Gill, A., Robinson, K.L., Umina, P.A., Ross, P.A., Zhan, D., Brown, C., Bell, N., MacMahon, A., Hoffmann, A.A., 2023. A diversity of endosymbionts across Australian aphids and their persistence in aphid cultures. Environ. Microbiol. 1462–2920, 16432. https://doi.org/10.1111/1462-2920.16432.