


RESEARCH

Open Access



Indirect and direct interactions between grain aphid and parasitoid in the presence of symbiont *Regiella insecticola*

Yue Man^{1†}, Delu Li^{1†}, Minghui Wang^{2†}, Zuqing Hu^{1*}, Jean-Luc Gatti³, Nicolas Desneux³, Peng Han² and Chen Luo^{1*} 

Abstract

Background Aphids often harbor bacterial symbionts that confer resistance to biotic and abiotic stress. Previous studies have primarily examined the direct effects of symbiont infection on parasitoid success but less on aphid population dynamics under indirect parasitic situations, for example when exposed to parasitoid wasp odor. Deciphering this type of indirect effect is essential for understanding dynamics of insect ecosystems and communities and to improve IPM success.

Methods We generated *Sitobion avenae* aphid clonal lines that are genetically identical but differ in *Regiella insecticola* infection. Then, the indirect odor effect of female parasitoid wasp *Aphidius gifuensis* (Ashmead), one of its major natural enemies in the fields, was examined on the aphid lines fitness using different parasitoid densities. With these lines we also tested the direct effect of symbiont presence on aphid resistance against different parasitoid densities.

Results Our study found fitness costs for the aphid line hosting *Regiella*, mainly via an increase in the development time and a reduction in population increase rate. Some of these fitness traits were influenced by the indirect exposure to parasitoid wasp odor with a density effect. Presence of the symbiont also reduced *A. gifuensis* parasitic success, increased the wasp development time and decreased its emergence weight with low effect of the parasitoid density used for parasitism.

Conclusions These results showed that aphid population dynamic was mainly affected by the symbiont presence, but not by parasitoid odor. Symbiont presence also protected aphid from parasitism and affected parasitoid offspring weight and hence their future individual fertility and fitness.

Keywords Parasitoid indirect effect, Symbiont-conferred resistance, Population dynamic, Aphids, *Aphidius gifuensis*

[†]Yue Man, Delu Li, Minghui Wang contributed equally to this study.

*Correspondence:

Zuqing Hu

huzuqing@nwsuaf.edu.cn

Chen Luo

chen.luo@nwsuaf.edu.cn; lczhibao@163.com

Full list of author information is available at the end of the article



Background

Wheat is one of the most important food resources for human beings, and its yield loss is mainly dependent on the magnitudes of pest attack and plant virus infection. Piercing-sucking insect pests can suck wheat phloem sap but also transmit many plant viruses, which seriously affect the wheat growth and reduce wheat production (Li et al. 2019; Luo et al. 2020a; Zogli et al. 2020). Evidences have shown that parasitoids may be the most important agents for controlling piercing-sucking pests (Schmidt et al. 2003; Stell et al. 2022). However, parasitoid's hosts often shelter endosymbionts that confer biotic and abiotic resistance (Desneux et al. 2018; Liu et al. 2022). For example, emerging evidences have shown that some of them can protect against parasitic wasps in aphids (Oliver et al. 2010; Monticelli et al. 2019a; Vorburger 2022). It is thus important to understand the role(s) of these symbionts in the efficiency of biological pest control (Käch et al. 2018; Attia et al. 2022).

Aphid is one of the most serious piercing-sucking agricultural pests on wheat plants worldwide (Li et al. 2020, Aradottir and Crespo-Herrera 2021). Most aphids live in obligatory symbiosis with the intracellular bacterium *Buchnera aphidicola* (Sochard et al. 2021), which synthesizes some essential amino acids and vitamins for the host survival and reproduction (Perreau and Moran 2022). In addition, some aphid individuals can also host one or more facultative symbionts, but their association varies from free association to co-obligation with intermediate stages of dependence (Henry et al. 2013; Guyomar et al. 2018). Until now, at least 9 different facultative symbionts have been described from aphids caught in the fields, the most common being *Hamiltonella defensa*, *Regiella insecticola*, and *Serratia symbiotica*, while *Rickettsiella viridis*, *Candidatus Fukatsuia symbiotica*, *Rickettsia*, *Spiroplasma*, *Wolbachia* and *Arsenophonus* are rare in most aphid populations (Augustinos et al. 2011; Guyomar et al. 2018; Cariou et al. 2018). In general, facultative symbionts are not necessary for the growth and reproduction of host aphids, but they could strongly affect the resistance (i.e., direct effects) to natural enemies (Scarborough et al. 2005; Zytynska et al. 2021; Luo et al. 2022; Frago and Zytynska 2023). Interestingly, emerging evidences suggested that indirect interactions (e.g., natural enemy-mediated effects in insect behavior and physiology by odors) are equally or more significant in the change of host population dynamic than the direct effect (Hermann and Landis 2017; Luo et al. 2022). In addition, the odor-mediated effects of natural enemies often occur in the field and may further involve other trophic organisms. Therefore, deciphering this type of indirect effect is essential for the understanding dynamics of insect ecosystems and communities (Ives and Carpenter 2007; Luo

et al. 2022). A new literature has shown that compared to without exposure to the predator *Harmonia axyridis*, the effect of symbiont *Regiella* on aphid population increase would be compromised under the indirect odor effect (Luo et al. 2022). However, wasp odor-mediated effects on the behavior and physiology of aphid carrying or not the symbiont have not been well documented.

During previous surveys of grain aphid *Sitobion avenae* populations from different locations in China, we found that most of these populations were infected by *Regiella* (Luo et al. 2016; Hu et al. 2020). Some studies have suggested that the presence of *Regiella* could have a fitness cost for host aphids, but help to resist parasitoids (Von et al. 2008; Luo et al. 2020a; Vorburger and Goukov 2011). Evidence for this trade-off is limited under the indirect interactions in aphid-parasitoid communities, although a study has shown that symbionts can protect aphids from parasitic wasps by attenuating herbivore-induced plant volatiles (Frago et al. 2017). Besides that, some evidences also suggested that the odor-mediated effect on insects could be mediated by natural enemy density component (Muller and Godfray 1999; Uriel et al. 2021). Here, we studied, under different densities of parasitoids, the effects of *Regiella* on: (1) aphid population dynamics under the odor-mediated situation of the parasitoid *Aphidius gifuensis*, and (2) resistance levels against this parasitoid. These results are expected to broaden the ecological roles of *Regiella*, and heighten the significance of facultative symbionts in biological pest control.

Materials and methods

Biological materials

To explore the biological effects of the *Regiella insecticola* symbiont, it was important to generate aphid strains that were genetically identical and differed only in *Regiella* infection. Two clone lines derived from the *Sitobion avenae* YL₂₇ were used in the current study. The natural YL₂₇-NA clone line, devoid of facultative symbionts, was derived from a single parthenogenetic aphid female, which was collected from a winter wheat field on the university research farm (Northwest A&F University, Yangling, China) in May 2019. The YL₂₇-Ri was obtained by the microinjection of body fluids of *Regiella*-infected *S. avenae* clone YP-Ri (Luo et al. 2022) to YL₂₇-NA (Chen and Purcell 1997). This new clone line artificially harboring *Regiella* was established after confirming the presence of the symbiont at the 10th generations (Additional file 1: Table S1). All these aphid lines were fed on wheat seedlings (*Triticum aestivum* L. cv. 'Aikang 58') and maintained in a climatic room (20 ± 1 °C, RH of 75%, LD 16: 8 h). During the study, the two YL₂₇ clone lines were regularly screened by PCR method (Monticelli et al. 2019b) to confirm

the presence/absence of *Regiella*, and have been maintained for over 1 year prior to the present study.

The parasitoid *Aphidius gifuensis*, maintained on the symbiont-free *S. avenae* clone Linyi-NA (Luo et al. 2017a) under the laboratory setting, was collected from a wheat field of the university experimental farm (Northwest A&F University, Yangling, China) in June 2014. Adult wasps were maintained in separate cages (side length = 25 cm) with cotton balls wetted with a honey solution (honey/water = 1/10). Four-day-old female wasps assumed to be mated were used in the experiment. Thirty minutes before the parasitism assays, the female wasp was exposed to uninfected 2nd-instar aphids in a Petri dish and only the wasp that oviposited within 5 min were used in the experiments (Luo et al. 2020b).

Effect of female wasp parasitoid odor on aphid fitness

To obtain synchronized aphid individuals for each aphid line, 10 adult wingless aphids (11 days old) were transferred to a new plant to reproduce for 12 h. Then, 30 aphid offspring were collected and transferred to an aerated transparent container (Ø 6 cm and 25 cm height) with a 2-leaf stage wheat. After 6 h of adaptation, a 50 ml of conical centrifugal tube, with a 100-micron nylon filter mesh at the top for ventilation, containing parasitoids (1, 3 or 5 individuals; 0 = empty tube used as the control; in order to avoid the odor interplay of different parasitoids, the treatment groups and the control group were kept in the transparent Perspex cages and over five meters apart from each other) was introduced for 6 h to expose aphids to the wasp odor. Thereafter, the aphids were individually transferred into a new two-leaf stage wheat seedling in aerated transparent cages. Developmental times of the 4 nymphal stages (DT1 ~ DT4), adult pre-reproductive period (APRP; the duration from adult emergence to the first reproduction) and total pre-reproductive period (TPRP; the duration from birth to the first reproduction; only reproducing aphids were considered in the following analysis) were recorded. In addition, the offspring number and mortality event of each aphid were also recorded daily until all died. During this assay, all offspring were removed every day to maintain a uniform reproduction environment. Each treatment was performed using four groups for each clone lines, with 30 aphids in each group. Finally, based on the aphid fitness parameters, a TIMING-MS Chart was used to predict the population growth of the *S. avenae* lines. For this prediction, we assumed unlimited growth with an initial population of 10 pairs of newly emerged *S. avenae* nymphs (Chi et al. 2022a).

Effect of symbiont on aphid parasitism

For each clonal line, thirty 4-day-old synchronized nymphs (2nd-instar) were transferred on a two-leaf stage wheat seedling in a transparent ventilated container. After 18 h, female wasps (1, 3 or 5) were introduced into the container and left 6 h for parasitism. Four days later, the 30 aphids were individually placed into Petri dishes (Ø 9 cm), containing a fresh wheat leaf blade that was replaced every 3 days (Luo et al. 2020b). During the following ~ 20 days, the numbers of successfully parasitized individuals (mummies), emerged adult wasps and wasp development time in aphids (from the day of parasitoid removal until the emergence of wasp offspring) were recorded. The emerged wasps were also weighed within 24 h (Mettler Toledo XS3DU; Mettler Toledo GmbH, Switzerland). Twelve biological replicates were performed for this study.

Statistical analyses

The data of the aphid fitness were analyzed by using the computer program TWSEX-MS Chart (Chi et al. 2022a; Chi et al. 2022b) to obtain aphid population parameters (i.e., intrinsic rate of increase, finite rate of increase, net reproductive rate and mean generation time) and aphid development times (DT1 ~ DT4, APRP and TPRP). In brief, the intrinsic rate of increase (r) was calculated by the Euler-Lotka equation ($\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1$) based on the iterative bisection method, showing a potential of population growth, where l_x is the age-specific survival rate, m_x is the age-specific fecundity, x is age interval; the finite rate of increase (λ) was equal to e^r ; the net reproductive rate ($R_0 = \sum_{x=0}^{\infty} l_x m_x$) illustrated a mean number of aphid offspring produced during one life cycle; mean generation time ($T = \frac{\ln R_0}{r}$) showed a time that aphid population reach to R_0 -times under a stable age-stage distribution condition. Parasitism rate in the study was measured by the number of mummified aphids divided by the number of whole treated aphids (30 aphids), and emergence rate was measured by the number of emerging parasitoids divided by the number of mummified aphids. Containers that had more than 5 missing aphids or had suffered no parasitism have been discarded during the statistical analysis (i.e., two repetitions were removed under the three parasitoid densities).

Factorial two-way analyses of variance were done to test the effects of parasitoid density, symbiont presence or absence and their interaction on the fitness and parasitism parameters calculated in the study. No interaction was found between (Parasitoid density × Symbiont) for all these traits (see Additional file 1: Table S2). Then, based on the analysis results above, the population parameters (i.e., r , λ , R_0 , T), aphid development times, weight of

emerged wasps and development time of the parasitoid offspring were analyzed using general linear models after validation of the normal distribution of the dependent variable. In addition, effects of the presence of the symbiont on the parasitism rate and emergence rate were analyzed by generalized linear modeling with a quasi-binomial error distribution (link=logit), which considers any over-dispersion in the data. The package “multcomp” was used to perform multiple comparisons using Tukey’s contrasts in the study. All statistics were done with R version 3.4.3.

Results

Effect of presence of symbiont on aphid fitness parameters under the indirect odor effect

The fitness traits of symbiotic aphids were compared with those of the symbiont-free aphids without (0) or after the exposure to the parasitoid odors using different parasitoid densities (1, 3 or 5 individuals). For the population parameters (Fig. 1, Table 1), in the absence of parasitoid

exposition, compared to symbiont-free aphids, symbiont-infected aphids showed a reduction in the intrinsic rate of increase (r) and the finite rate of increase (λ), but had an increase on the mean generation time (T). Under the 1, 3, or 5 wasps odor exposition, we found a similar result between infected and uninfected aphids on the three population traits. The net reproductive rate (R_0) was significantly reduced under the 1 or 5 wasps density in the infected aphids. For the development times (Tables 1, 2), in the absence of parasitoid exposition, compared with symbiont-free aphids, symbiont-infected aphids had a significant increase on the development time of 1st and 2nd-instars (DT1, DT2) as well as the adult pre-reproductive period (APRP). A similar result on the three traits between infected and uninfected aphids was also found under the 1, 3, or 5 wasps density. A significant increase of the DT3, DT4 and TPRP (total pre-reproductive period) of the infected aphids was observed with the 3 wasps density, and a clear increase on DT3 and TPRP was examined with the 5 wasp density and the

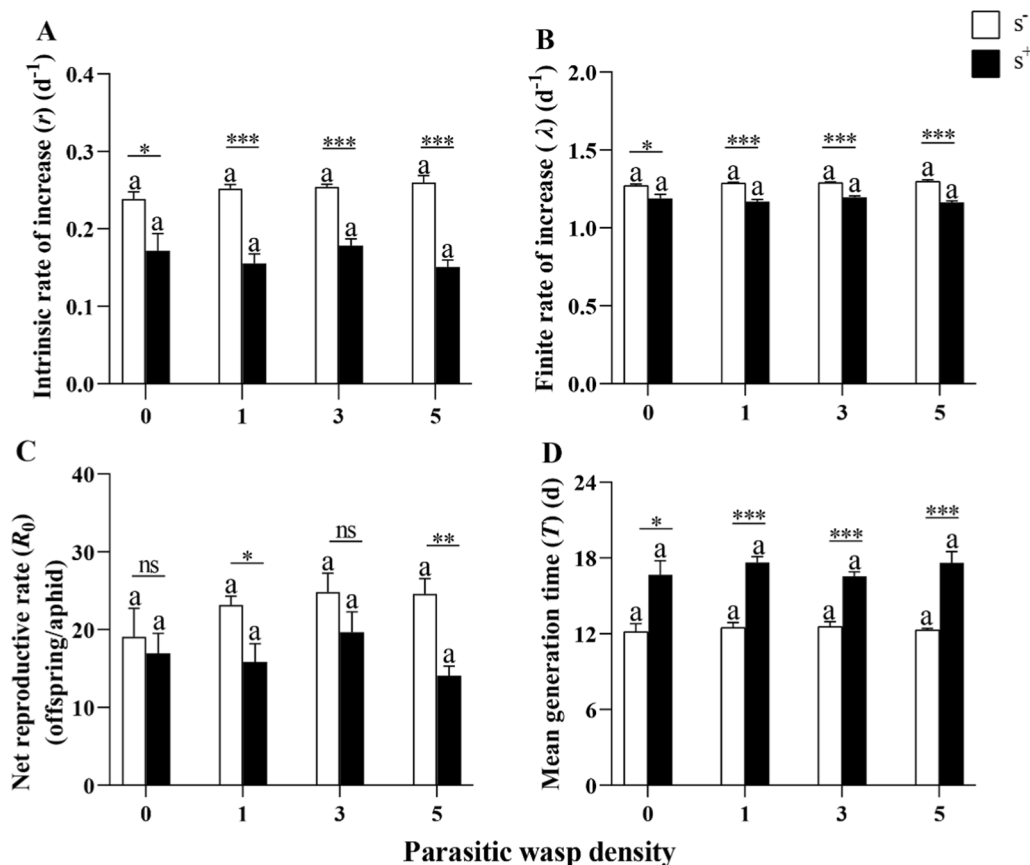


Fig. 1 Population parameters of the *Regiella*-infected and the symbiont-free *S. avenae* lines submitted to *A. gifuensis* odor. The different letters indicated significant difference ($P < 0.05$) between symbiont-free (S⁻; white column) or symbiont-infected (S⁺; black column) aphids facing odor of different density of *A. gifuensis*. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (significant difference between aphid lines for the same densities of *A. gifuensis*, ns: no significant difference). Each value is the Mean \pm SEM

Table 1 Statistical analyses of the effects of *Regiella* presence on aphid populational traits under different densities of *A. gifuensis*. Table indicates the intrinsic rate of increase (*r*), finite rate of increase (λ), net reproductive rate (R_0), mean generation time (*T*), and the developmental times of 1st to 4th instar nymphs (DT1~DT4), adult pre-oviposition period (APRP), total pre-oviposition period (TPRP) of host aphids across different *A. gifuensis* densities (0, 1, 3, 5 parasitoids)

Variance source	df	0 wasp FP	1 wasp FP	3 wasps FP	5 wasps FP
<i>r</i>	1	7.857 < 0.05	50.122 < 0.001	63.445 < 0.001	73.459 < 0.001
λ	1	7.898 < 0.05	53.900 < 0.001	66.930 < 0.001	73.378 < 0.001
R_0	1	0.220 0.656	7.852 < 0.05	2.043 0.203	20.323 < 0.01
<i>T</i>	1	12.350 < 0.05	71.746 < 0.001	60.665 < 0.001	34.613 < 0.001
DT1	1	25.581 < 0.001	39.477 < 0.001	29.053 < 0.001	34.148 < 0.001
DT2	1	11.802 < 0.001	7.226 < 0.01	9.909 < 0.01	12.531 < 0.001
DT3	1	0.039 0.844	1.712 0.195	7.737 < 0.01	4.590 < 0.05
DT4	1	0.307 0.581	0.000 1.000	4.170 < 0.05	0.723 0.398
APRP	1	5.443 < 0.05	4.606 < 0.05	16.532 < 0.001	6.589 < 0.05
TPRP	1	4.016 < 0.05	1.182 0.280	9.665 < 0.01	1.776 0.186

Table 2 The effect of *Regiella* on the development times of host aphids under different densities of *A. gifuensis* (i.e., 0, 1, 3, 5 parasitoids). Days; Mean \pm SEM

Lines	Life-history traits					
	DT1	DT2	DT3	DT4	APRP	TPRP
YL ₂₇ -NA (0)	2.08 \pm 0.10Ab	1.55 \pm 0.10Ab	1.92 \pm 0.26Aa	1.80 \pm 0.17Aa	0.85 \pm 0.08Ab	6.40 \pm 0.53Ab
YL ₂₇ -Ri (0)	3.55 \pm 0.27Aa	2.40 \pm 0.23Aa	1.85 \pm 0.20Aa	1.95 \pm 0.22Aa	1.40 \pm 0.22Aa	8.68 \pm 1.01Aa
YL ₂₇ -NA (1)	2.05 \pm 0.18Ab	1.53 \pm 0.09Ab	1.55 \pm 0.09Aa	1.95 \pm 0.12Aa	1.00 \pm 0.07Ab	7.55 \pm 0.43Aa
YL ₂₇ -Ri (1)	4.13 \pm 0.29Aa	2.25 \pm 0.25Aa	1.95 \pm 0.29Aa	1.95 \pm 0.24Aa	1.65 \pm 0.29Aa	8.88 \pm 1.14Aa
YL ₂₇ -NA (3)	2.03 \pm 0.09Ab	1.73 \pm 0.09Ab	1.58 \pm 0.10Ab	1.83 \pm 0.087Ab	1.03 \pm 0.06Ab	7.78 \pm 0.31Ab
YL ₂₇ -Ri (3)	3.55 \pm 0.27Aa	2.58 \pm 0.25Aa	2.55 \pm 0.34Aa	2.28 \pm 0.20Aa	2.23 \pm 0.29Aa	11.13 \pm 1.03Aa
YL ₂₇ -NA (5)	1.77 \pm 0.10Ab	1.55 \pm 0.11Ab	1.45 \pm 0.09Ab	1.95 \pm 0.08Aa	1.05 \pm 0.06Ab	7.55 \pm 0.30Aa
YL ₂₇ -Ri (5)	3.62 \pm 0.30Aa	2.73 \pm 0.31Aa	1.93 \pm 0.20Aa	2.30 \pm 0.40Aa	1.73 \pm 0.26Aa	9.05 \pm 1.08Aa

Note: The capital letter indicated significant difference of symbiont-free or symbiont-infected aphids among different densities of *A. gifuensis* at $P < 0.05$ level. The lowercase letter indicated significant difference between symbiont-free and symbiont-infected aphids facing the same densities of *A. gifuensis* at $P < 0.05$ level

without-exposition to the parasitoid odors, respectively. Overall, the presence of the symbiont had an adverse effect on aphid population increase.

Effect of presence of symbiont on aphid parasitism under the different parasitoid density

The parasitism traits of infected aphids were compared with those of the uninfected aphids with different parasitoid density (1, 3 or 5 individuals) (Fig. 2). When parasitized by 1 female wasp, compared to symbiont-free aphids, infected aphids showed a lower parasitism rate (number of mummies; $\chi^2 = 57.622$, $df = 1$, $P < 0.001$), an increase on the parasitoid offspring development time ($F = 44.698$, $df = 1$, $P < 0.001$) and a lower weight of emerged parasitoid wasps ($F = 55.495$, $df = 1$, $P < 0.001$). A similar result on these parameters between infected and uninfected aphids was also found when 3 or 5 parasitoids were used (parasitism rate: $\chi^2 = 68.579$, $df = 1$, $P < 0.001$;

$\chi^2 = 75.061$, $df = 1$, $P < 0.001$; the weight: $F = 43.561$, $df = 1$, $P < 0.001$; $F = 94.097$, $df = 1$, $P < 0.001$; the development time: $F = 26.793$, $df = 1$, $P < 0.001$; $F = 18.792$, $df = 1$, $P < 0.001$, respectively). However, for the emergence rate, measured by the ratio of emerged parasitoids from the mummies, no difference was observed under the one density ($\chi^2 = 0.098$, $df = 1$, $P = 0.754$), while there was a significant decrease in the presence of *Regiella* with 3 or 5 parasitoids ($\chi^2 = 20.667$, $df = 1$, $P < 0.001$; $\chi^2 = 4.808$, $df = 1$, $P < 0.001$, respectively).

Effects of parasitoid wasp density on fitness and parasitism of the uninfected or infected aphids

The traits of the uninfected or infected aphids were compared among different parasitoid wasp density. For the fitness traits (see capital letter in Table 2), in the uninfected aphids (YL₂₇-NA), no difference on

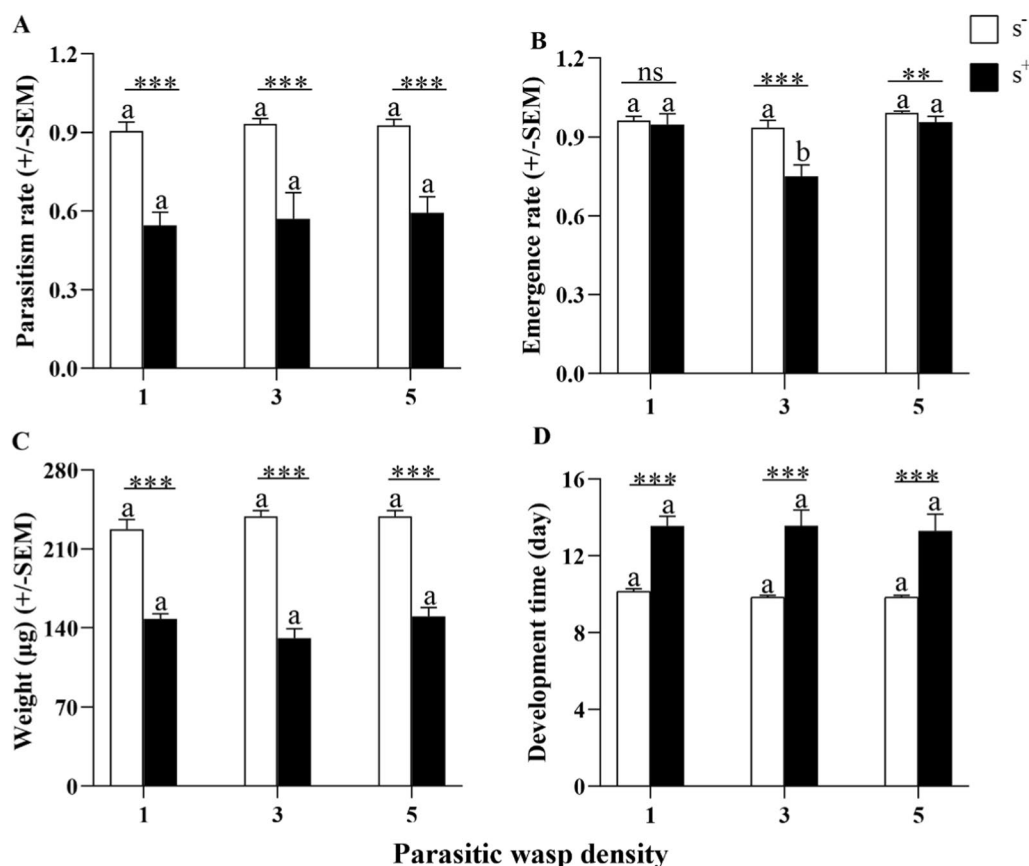


Fig. 2 Effects of *Regiella* infection on the parasitism rate (A), emergence rate (B), weight of emerged wasp (C) and development times of *A. gifuensis* (D) under different densities of *A. gifuensis*. The different letters indicated significant difference ($P < 0.05$) for symbiont-free (S-; white column) or symbiont-infected (S+; black column) aphids among different densities of *A. gifuensis*. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (significant difference between the two aphid lines for the same densities of *A. gifuensis*, ns: no significant difference). Each value is the Mean \pm SEM

the population parameters (r : $P = 0.244$; λ : $P = 0.243$; R_0 : $P = 0.371$; T : $P = 0.894$) and the development time traits (DT1: $P = 0.294$; DT2: $P = 0.462$; DT3: $P = 0.161$; DT4: $P = 0.715$; APRP: $P = 0.149$; TPRP: $P = 0.073$) was observed among the different parasitoid densities (0, 1, 3 and 5 individuals). Similar results were obtained for these traits (r : $P = 0.487$; λ : $P = 0.485$; R_0 : $P = 0.396$; T : $P = 0.635$; DT1: $P = 0.402$; DT2: $P = 0.606$; DT3: $P = 0.215$; DT4: $P = 0.688$; APRP: $P = 0.175$; TPRP: $P = 0.334$) in the infected aphids (YL₂₇-Ri) among the different parasitoid densities. For the parasitism traits (see letters in Fig. 2), there also was no difference for the uninfected or infected aphids on the parasitism rate ($P = 0.445, 0.752$), the weight of emerged parasitoid wasp ($P = 0.115, 0.165$) and the development time of parasitoid offspring ($P = 0.180, 0.958$) among different parasitoid densities (1, 3 and 5 individuals), except a reduction on the emergence rate of infected aphids at the medium density ($P < 0.01$).

Discussion

Symbiont-mediated trade-offs between the fitness cost and resistance have been widely reported in insects, but most focused on the model pea aphids (Zytyńska et al. 2021; Vorburger 2022). In addition, the effects of the density of parasitoid on aphid fitness and resistance were little known, although the density effect is common in nature and is important to understand community dynamics (Muller and Godfray 1999). Since the grain aphid *Sitobion avenae* is the most devastating pest for winter wheat in China (Xu et al. 2011; Jiang et al. 2019) and *Regiella* is widely present in *S. avenae* individuals in this country (Li et al. 2014; Luo et al. 2016; Hu et al. 2020), we investigated whether the presence of this symbiont could induce a trade-off between *S. avenae* fitness cost and resistance to *A. gifuensis*, a biological control agent widely used in China, across different parasitoid densities.

For symbiont-infected or symbiont-free aphids, the presence of different wasp density nearly had no impact

on traits of aphid fitness and resistance. Earlier studies also suggested that the parasitoid density had no effect on offspring numbers and the timing of peak reproduction of aphids across four experimental generations (Uriel et al. 2021). These results may suggest that aphid fitness/phenotypes were independent of the parasitoid in the range of density tested, but the conclusion should be viewed with caution for several reasons. For example, the setup of parasitoid density gradient in these studies may hardly induce a significant difference on the quantity of volatiles, although a previous study suggested that there was a significant ladybeetle-odor effect under the low density on aphid population dynamic (Luo et al. 2022). In addition, some studies also showed that in the presence of parasitoids, different aphid species would have various behavioral responses. For example, apterous pea aphids *Acyrtosiphon pisum* would produce alate offspring (Sloggett et al. 2002), which generally had lower reproductive output, whereas soybean aphids *Aphis glycines* produced offspring with greater reproductive output (Kaiser 2017). All these findings may suggest that the odor quantity and aphid species should be considered when exploring the indirect effect of parasitoids on insect fitness.

The results of aphid population parameters indicated that *Regiella* infection results in a significant decrease on the intrinsic rate of increase (r) across different parasitoid densities (1, 3 and 5 individuals), the most important parameter for evaluating the population growth potential of insects (Qayyum et al. 2018). Therefore, the adverse effect on the intrinsic rate of increase suggested that *Regiella* infection was significantly disadvantageous for the increase of aphid population number and was not wasp density-dependent, since a small change of this parameter could cause a significant change in insect population numbers (Qayyum et al. 2018; Chi et al. 2022a; Chi et al. 2022b). This negative impact on aphid population growth rate was confirmed by the result of the population projection model in the study (Additional file 1: Fig. S1). In addition, this notion was also verified by the results of the net reproductive rate (R_0) and the finite rate of increase (λ) of the infected aphids, which were lower than that of their corresponding symbiont-free aphid line. It should be noted that the λ value of either the symbiont-infected aphid or the symbiont-free line was greater than one, suggesting that the population studied may have increased continuously (Liang et al. 2021). How could the symbiont influence population dynamic of host aphid? One possible explanation could be an increase of the development time of 1st and 2nd-instars (DT1, DT2) as well as adult pre-reproductive period (APRP) of symbiont-infected *S. avenae* observed in the study, which is consistent with previous reports (Laughton et al.

2014; Luo et al. 2017b; Luo et al. 2020a). The increased development time may reduce or delay aphid reproduction but this could be counterbalanced by the benefit of an increase parasitoid resistance, suggesting a trade-off between resistance and fitness cost of aphids. In the field, symbiont-infected and symbiont-free aphids often occur side-by-side and are typical for real aphid populations, which are generally attributed to balancing selection (Oliver et al. 2014; Gimmi et al. 2023).

Indeed, the presence of symbiont reduced the parasitism success of wasps across different parasitoid densities. The conferred resistance of *Regiella* was confirmed by the clear reduction on the weight of emerged wasps and the emergence rate from symbiont-infected aphids. This result was expected, since previous reports have suggested that some *Regiella* strains should be regarded as a defensive symbiont in aphids that can adversely influence the normal development of parasitoid eggs/larvae by encoding pathogenicity factors such as RTX toxins (Vorburger et al. 2010; Hansen et al. 2012; Luo et al. 2022; Vorburger 2022). In addition, since previous studies have suggested that the presence of endosymbionts could impact the cellular and humoral immunity of host aphids (Schmitz et al. 2012; Luo et al. 2021), we here propose that parasitism success may also be related with host immune responses generated by the presence of *Regiella*.

Conclusions

Overall, this study documented that *Regiella insecticola* infection would lower aphid population growth and increase the resistance to the parasitoid *Aphidius gifuensis* and that these parameters were not highly affected by the parasitoid densities. The results suggest that there could be a possible compromise between the fitness cost of aphids and their resistance, which could offer a new outlook on the utilization of parasitoids in biological pest control by incorporating the effects of symbionts. This, in turn, could aid in the enhancement of integrated pest management (IPM) by reducing the reliance on pesticides and minimizing the negative impact on non-target insects. (Desneux et al. 2018).

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s43170-023-00202-1>.

Additional file 1: Table S1. PCR of symbionts. We tested the aphid lines for the different known symbionts classically reported in aphids as previously described (Monticelli et al. 2019b). (−) symbiont absence; (+) symbiont presence. **Table S2.** Effects of densities of parasitoids, presence or absence of symbiont and their interaction on the intrinsic rate of increase (r), finite rate of increase (λ), net reproductive rate (R_0), mean generation time (T), and developmental times of 1st to 4th instar nymphs (DT1~DT4), adult pre-oviposition period (APRP), total pre-oviposition period (TPRP), as well as parasitism rate, emergence rate, emergence

time and wasp weight of aphid clone lines. Factorial ANOVAs were performed. **Figure S1.** Simulated adult population growth for 60 days of the symbiont-free *S. miscanthi* line (A) and the *Regiella*-infected line (B) under different densities of *A. gifuensis* parasitoid (0, 1, 3, 5).

Acknowledgements

The authors are grateful to the Avaguri for collecting aphids and mummies in the field.

Author contributions

CL, ZQH, PH, ND conceptualized the idea, YM, DLL, MHW carried out the investigations, YM, DLL analyzed the data and wrote the first draft manuscript, CL, ZQH, PH, JLG, ND reviewed the manuscript. All authors read and approved the final manuscript.

Funding

This research was funded by the National Natural Science Foundation of China (32202297), the China Postdoctoral Science Foundation funded project (2022M712164), and Chinese Universities Scientific Fund (2452021045).

Availability of data and materials

The data presented in this study are available within the article and its supplementary information file.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

Nicolas Desneux is senior editor of *CABI Agriculture and Bioscience* and was not involved in the review process and decisions related to this manuscript.

Author details

¹State Key Laboratory of Crop Stress Biology for Arid Areas, Key Laboratory of Plant Protection Resources and Pest Management of Ministry of Education, Key Laboratory of Integrated Pest Management On the Loess Plateau of Ministry of Agriculture and Rural Affairs, College of Plant Protection, Northwest A&F University, Yangling 712100, Shaanxi, China. ²Institute of Biodiversity, School of Ecology and Environmental Science, Yunnan University, Kunming 650500, China. ³Université Côte d'Azur, INRAE, CNRS, UMR-ISA, 06000 Nice, France.

Received: 4 July 2023 Accepted: 8 December 2023

Published online: 19 December 2023

References

- Aradottir GI, Crespo-Herrera L. Host plant resistance in wheat to barley yellow dwarf viruses and their aphid vectors: a review. *Curr Opin Insect Sci.* 2021;45:59–68.
- Attia S, Renoz F, Pons I, Louâpre P, Foray V, Piedra JM, et al. The aphid facultative symbiont *Serratia symbiotica* influences the foraging behaviors and the life-history traits of the parasitoid *Aphidius ervi*. *Entomol Gen.* 2022;42:21–33.
- Augustinos AA, Santos-Garcia D, Dionyssopoulou E, Moreira M, Papapanagiotou A, Scarvelakis M, Doudoumis V, Ramos S, Aguiar AF, Borges PA, Khadem M. Detection and characterization of *Wolbachia* infections in natural populations of aphids: is the hidden diversity fully unraveled? *PLoS ONE.* 2011;6: e28695.
- Cariou M, Ribière C, Morlière S, Gauthier JP, Simon JC, Peyret P, Charlat S. Comparing 16S rDNA amplicon sequencing and hybridization capture for pea aphid microbiota diversity analysis. *BMC Res Notes.* 2018;11:461.
- Chen D, Purcell AH. Occurrence and transmission of facultative endosymbionts in aphids. *Curr Microbiol.* 1997;34:220–5.
- Chi H, Güncan A, Kavousi A, Gharakhani G, Atlihan R, Özgökçe MS, et al. TWSEX-MSChart: the key tool for life table research and education. *Entomol Gen.* 2022a;42:845–9.
- Chi H, Kara H, Özgökçe MS, Atlihan R, Güncan A, Rişvanlı MR. Innovative application of set theory, Cartesian product, and multinomial theorem in demographic research. *Entomol Gen.* 2022b;42:863–74.
- Desneux N, Asplen MK, Brady CM, Heimpel GE, Hopper KR, Luo C, et al. Intraspecific variation in facultative symbiont infection among native and exotic pest populations: potential implications for biological control. *Biol Control.* 2018;116:27–35.
- Frago E, Zytynska S. Impact of herbivore symbionts on parasitoid foraging behaviour. *Curr Opin Insect Sci.* 2023;57: 101027.
- Frago E, Mala M, Weldegergis BT, Yang CJ, McLean A, Godfray H CJ, et al. Symbionts protect aphids from parasitic wasps by attenuating herbivore-induced plant volatiles. *Nat Commun.* 2017;8:1860.
- Gimmi E, Wallisch J, Vorburger C. Defensive symbiosis in the wild: Seasonal dynamics of parasitism risk and symbiont-conferred resistance. *Mol Ecol.* 2023;32:4063–77.
- Guyomar C, Legeai F, Jousselin E, Mougel C, Lemaitre C, Simon JC. Multi-scale characterization of symbiont diversity in the pea aphid complex through metagenomic approaches. *Microbiome.* 2018;6:181.
- Hansen, AK, Vorburger, C, Moran, NA. Genomic basis of endosymbiont-conferred protection against an insect parasitoid. *Genome Res.* 2012;22:106–14.
- Henry LM, Peccoud J, Simon JC, Hadfield JD, Maiden MJC, Ferrari J, Godfray H CJ. Horizontally transmitted symbionts and host colonization of ecological niches. *Curr Biol.* 2013;23:1713–7.
- Hermann SL, Landis DA. Scaling up our understanding of non-consumptive effects in insect systems. *Curr Opin Insect Sci.* 2017;20:54–60.
- Hu ZQ, Su D, Li DD, Tong ZQ, Zhang C, Zhang GS, Zhao HY, Luo C. Diversity of secondary endosymbionts among different geographical populations of the grain aphid, *Sitobion avenae* (Fabricius) (Hemiptera:Aphididae) in China. *Entomol Gen.* 2020;40:253–62.
- Ives AR, Carpenter SR. Stability and diversity of ecosystems. *Science.* 2007;317:58–62.
- Jiang X, Zhang Q, Qin YG, Yin H, Zhang SY, Li Q, Zhang Y, Fan J, Chen JL. A chromosome-level draft genome of the grain aphid *Sitobion miscanthi*. *GigaScience.* 2019;8:giz101.
- Käch H, Mathé-Hubert H, Dennis AB, Vorburger C. Rapid evolution of symbiont-mediated resistance compromises biological control of aphids by parasitoids. *Evol Appl.* 2018;11:220–30.
- Kaiser MC. Transgenerational fecundity compensation and post-parasitism reproduction by aphids in response to their parasitoids. PhD thesis, University of Minnesota. 2017.
- Laughton AM, Fan MH, Gerardo NM. The combined effects of bacterial symbionts and aging on life history traits in the pea aphid. *Acyrtosiphon Pisum Appl Environ Microbiol.* 2014;80:470–7.
- Li T, Xiao JH, Wu YQ, Huang DW. Diversity of bacterial symbionts in populations of *Sitobion miscanthi* (Hemiptera: Aphididae) in China. *Environ Entomol.* 2014;43:605–11.
- Li DD, Su D, Tong ZQ, Zhang C, Zhang G, Zhao HY, Hu ZQ. Virus-dependent and -independent responses of *Sitobion avenae* (Hemiptera: Aphididae) feeding on wheat infected by transmitted and nontransmitted viruses at transcriptomic level. *J Econ Entomol.* 2019;112:2067–76.
- Li DD, Zhang C, Tong ZQ, Su D, Zhang GS, Zhao HY, Hu ZQ. Transcriptome response comparison between vector and nonvector aphids after feeding on virus-infected wheat plants. *BMC Genomics.* 2020;21:638.
- Liang HY, Yang XM, Sun LJ, Zhao CD, Chi H, Zheng CY. Sublethal effect of spiroetramat on the life table and population growth of *Frankliniella occidentalis* (Thysanoptera: Thripidae). *Entomol Gen.* 2021;41:219–31.
- Liu B, Lu Y, Wan F, Gershenson J, Cheng D. Biological invasion of insects: the roles of microbes. *Entomol Gen.* 2022;42:851–61.
- Luo C, Monticelli LS, Meng LQ, Li DD, Fan JY, Zhao HY, Hu ZQ. Effect of the endosymbiont *Regiella insecticola* on an aphid parasitoid. *Entomol Gen.* 2017a;36:300–7.
- Luo C, Luo K, Meng L, Wan B, Zhao H, Hu ZQ. Ecological impact of a secondary bacterial symbiont on the clones of *Sitobion avenae* (Fabricius) (Hemiptera: Aphididae). *Sci Rep.* 2017b;7:40754.

- Luo C, Monticelli LS, Li DD, Ahmed SS, Pandharikar G, Zhao HY, Desneux N, Hu ZQ. Comparison of life-history traits and resistance for *Sitobion avenae* (Fabricius) harboring a facultative symbiont. *Entomol Gen.* 2020a;40:39–47.
- Luo C, Gatti JL, Monticelli LS, Poirié M, Desneux N, Zhao HY, Hu ZQ. An increased risk of parasitism mediated by the facultative symbiont *Regiella insecticola*. *J Pest Sci.* 2020b;93:737–45.
- Luo C, Belghazi M, Schmitz A, Lemauf S, Desneux N, Simon JC, Poirié M, Gatti JL. Hosting certain facultative symbionts modulate the phenoloxidase activity and immune response of the pea aphid *Acyrtosiphon pisum*. *Insect Sci.* 2021;28:1780–99.
- Luo C, Chai RR, Liu X, Dong Y, Desneux N, Feng Y, Hu ZQ. The facultative symbiont *Regiella insecticola* modulates non-consumptive and consumptive effects of *Harmonia axyridis* on host aphids. *Entomol Gen.* 2022;42:733–41.
- Luo C, Luo K, Hu ZQ, Tao YY, Zhao HY. The infection frequencies and dynamics of three secondary endosymbionts in the laboratory environments on *Sitobion avenae* (Fabricius) as determined by long PCR. *J Asia-Pac Entomol.* 2016;19:473–6.
- Monticelli LS, Outreman Y, Frago E, Desneux N. Impact of host endosymbionts on parasitoid host range—From mechanisms to communities. *Curr Opin Insect Sci.* 2019a;32:77–82.
- Monticelli LS, Nguyen LT, Amiens-Desneux E, Luo C, Lavoire AV, Gatti JL, Desneux N. The preference-performance relationship as a means of classifying parasitoids according to their specialization degree. *Evol Appl.* 2019b;12:1626–40.
- Muller CB, Godfray HCJ. Indirect interactions in aphid-parasitoid communities. *Res Popul Ecol.* 1999;41:93–106.
- Oliver KM, Degnan PH, Burke GR, Moran NA. Facultative symbionts in aphids and the horizontal transfer of ecologically important traits. *Annu Rev Entomol.* 2010;55:247–66.
- Oliver KM, Smith AH, Russell JA, Clay K. Defensive symbiosis in the real world—Advancing ecological studies of heritable, protective bacteria in aphids and beyond. *Funct Ecol.* 2014;28:341–55.
- Perreau J, Moran NA. Genetic innovations in animal-microbe symbioses. *Nat Rev Genet.* 2022;23:23–39.
- Qayyum A, Aziz A, Iftikhar A, Hafeez F, Atlihan R. Demographic parameters of *Lipaphis erysimi* (Hemiptera: Aphididae) on different cultivars of brassica vegetables. *J Econ Entomol.* 2018;111:1885–94.
- Scarborough CL, Ferrari J, Godfray HCJ. Aphid protected from pathogen by endosymbiont. *Science.* 2005;310:1781.
- Schmidt MH, Lauer A, Purtauf T, Thies C, Schaefer M, Tschardt T. Relative importance of predators and parasitoids for cereal aphid control. *P Roy Soc B-Biol Sci.* 2003;270:1905–9.
- Schmitz A, Anselme C, Ravallec M, Rebuf C, Simon JC, Gatti JL, Poirie M. The cellular immune response of the pea aphid to foreign intrusion and symbiotic challenge. *PLoS ONE.* 2012;7: e42114.
- Skaljic M, Kirfel P, Grotmann J, Vilcinskis A. Fitness costs of infection with *Serratia symbiotica* are associated with greater susceptibility to insecticides in the pea aphid *Acyrtosiphon pisum*. *Pest Manag Sci.* 2018;74:1829–36.
- Sloggett JJ, Weisser WW. Parasitoids induce production of the dispersal morph of the pea aphid. *Acyrtosiphon Pisum Oikos.* 2002;98:323–33.
- Sochard C, le Floch M, Anton S, Outreman Y, Simon JC. Limited influence of gain and loss of symbionts on host plant selection in specialized pea aphid genotypes. *Entomol Gen.* 2021;41:39–47.
- Stell E, Meiss H, Lasserre-Joulin F, Therond O. Towards predictions of interaction dynamics between cereal aphids and their natural enemies: a review. *Insects.* 2022;13:479.
- Uriel Y, Abram PK, Gries G. Parasitoid pressure does not elicit defensive polyphenism in the green peach aphid. *Ecol Eetomol.* 2021;46:668–76.
- Von BS, Ferrari J, Müller CB, Vorburger C. Genetic variation and covariation of susceptibility to parasitoids in the aphid *Myzus persicae*: no evidence for trade-offs. *P Roy Soc B-Biol Sci.* 2008;275:1089–94.
- Vorburger C. Defensive symbionts and the evolution of parasitoid host specialization. *Annu Rev Entomol.* 2022;67:329–46.
- Vorburger C, Gousskov A. Only helpful when required: a longevity cost of harbouring defensive symbionts. *J Evolution Biol.* 2011;24:1611–7.
- Vorburger C, Gehrler L, Rodriguez P. A strain of the bacterial symbiont *Regiella insecticola* protects aphids against parasitoids. *Biol Lett.* 2010;6:109–11.
- Xu ZH, Chen JL, Cheng DF, Yong LI, Frédéric F. Genetic variation among the geographic population of the grain aphid, *Sitobion avenae* (Hemiptera: Aphididae) in China inferred from mitochondrial *COI* gene sequence. *Agric Sci China.* 2011;10:1041–8.
- Zogli P, Pingault L, Grover S, Louis J. Ento(o)mics: the intersection of “omic” approaches to decipher plant defense against sap-sucking insect pests. *Curr Opin Plant Biol.* 2020;56:153–61.
- Zytynska SE, Tighiouart K, Frago E. The benefits and costs of hosting facultative symbionts in plant-sucking insects: a meta-analysis. *Mol Ecol.* 2021;30:2483–94.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

