

Reviewed Preprint

v1 • September 15, 2026

Not revised

✉ For correspondence:

xigu2@unimelb.edu.au

Competing interests: No

competing interests declared

Funding: See [page 18](#)

Reviewing editor: Sergio Rasman, University of Neuchâtel, Switzerland

© 2026, Gu et al. This article is distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use and redistribution provided that the original author and source are credited.

Novel but stable endosymbionts have contrasting effects on aphid dispersal and plant feeding damage in the cereal pest *Diuraphis noxia*

Xinyue Gu¹ ✉, Alex Gill¹, Qiong Yang¹, Perran A Ross^{1,2}, Lucy Hayward¹, Monica Stelmach¹, Paul A Umina^{1,3}, Sheik Nadeem Elahee Doomun⁴, Mel Berran¹, Lauren Coakley¹, Sonia Sharma¹, Ary A Hoffmann¹

¹Pest and Environmental Adaptation Research Group, School of BioSciences, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Parkville, Australia • ²Section for Bioscience and Engineering, Department of Chemistry and Bioscience, Aalborg University, Aalborg, Denmark • ³Cesar Australia, Brunswick, Australia •

⁴Metabolomics Australia, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Parkville, Australia

eLife Assessment

This **important** study investigates how experimentally introducing two facultative bacterial endosymbionts into the Russian wheat aphid, *Diuraphis noxia*, affects aphid performance, dispersal, and damage to cereal host plants. The authors provide **solid** evidence that the two symbionts can generate contrasting phenotypes: *Rickettsiella* increases plant damage while reducing wing formation and dispersal, whereas *Regiella* reduces aphid population growth and feeding damage. The successful establishment and stable transmission of these novel symbiont-host associations, combined with experiments spanning individual, whole-plant, population and mesocosm scales, are notable strengths of the work; however, the mechanisms underlying these effects remain unresolved, evidence for horizontal transmission is indirect, and some population-level conclusions rely on relatively small sample sizes or effects that are not consistently detected across time points, and therefore the potential application of these findings to pest management remains promising but speculative. The study will be of broad interest to researchers working on insect symbiosis, plant-insect interactions, and biologically based approaches to pest management.

<https://doi.org/10.7554/eLife.111255.1.sa3>

Abstract

Endosymbiotic bacteria can affect many ecological attributes of their insect hosts, including (in herbivorous insects) how insects interact with plants where they feed. This raises the issue of whether deliberate endosymbiont introductions could be used to decrease crop damage caused by insect pests. Here we investigate how transinfecting *Rickettsiella viridis* and *Regiella insecticola* endosymbionts into a novel pest aphid host, the Russian wheat aphid (*Diuraphis noxia*), influences population growth, alate production, dispersal ability and crop damage. Both the *Rickettsiella* (originating from pea aphids) and *Regiella* (from green peach aphids) were stably maintained in their new host where they had contrasting effects. *Rickettsiella* increased the severity of aphid damage on wheat and barley, resulting in greater leaf loss, chlorotic streaking, and higher aphid populations, whereas *Regiella* reduced aphid population growth and the severity of feeding damage by aphids. Their effects on dispersal morphology also differed: *Regiella* had no detectable impact on alate incidence, while *Rickettsiella* consistently suppressed wing formation in small cages, and in larger mesocosms with multiple wheat plants this endosymbiont suppressed

dispersal. Endosymbiont-mediated changes in feeding damage did not involve the main plant immune response pathways: transinfected and wild type aphids induced similar levels of jasmonic acid, jasmonic acid-isoleucine, and salicylic acid in plant tissues, even though these plant defenses were strongly activated during aphid feeding. Novel endosymbionts can therefore modulate the severity of plant feeding damage by aphids as well as influencing aphid dispersal. Potential applications in controlling pest *D. noxia* populations are discussed.

Significance statement

Endosymbiotic bacteria that live within insect cells can have wide-ranging effects on the reproduction and fitness of their insect hosts in different environments. In herbivorous insects this includes effects on host plant use. Here we test if novel endosymbionts in a pest aphid, the Russian wheat aphid, might be used to decrease crop damage and dispersal. We show that the damage caused to wheat and barley plants from aphid feeding is modulated by novel but stably transmitted introduced endosymbionts. One endosymbiont (*Rickettsiella*) increased the severity of damage but decreased aphid dispersal, while another (*Regiella*) decreased damage severity without impacting dispersal. These contrasting effects may be associated with changes in aphid population growth and wing formation but were not linked to key plant immune response pathways. We discuss implications of these findings for using endosymbionts in agricultural pest management.

Introduction

Endosymbionts are heritable bacteria that reside within the cells of their hosts, occurring in more than half of all insect species. They have strong effects on insect phenotypes and can play important roles in shaping insect adaptation and evolutionary trajectories (1–3). Endosymbionts can modulate a range of biological aspects in their host insects including nutrient acquisition (4, 5), thermal tolerance (6–9), reproduction (10–12), resistance to pathogens and natural enemies (13, 14), and plant virus acquisition and transmission (15) thereby influencing the population dynamics of their insect hosts and species interactions.

Beyond influencing insect biology, endosymbionts may also modulate plant defense pathways, including those mediated by salicylic acid (SA) and jasmonic acid (JA), which are central plant responses to herbivory. For instance, in *Bemisia tabaci*, *Rickettsia* endosymbionts transferred into plants activate SA signalling while suppressing JA responses, enhancing herbivore fitness but increasing plant resistance to fungal and viral pathogens (16). This underscores their effects on multitrophic networks involving insects, microbial symbionts, host plants, and other associated pathogens. To date, most studies have focussed on two components, such as endosymbiont–insect or insect–plant interactions, while relatively few have directly examined how all three interact together.

Aphids (Hemiptera: Aphididae) are among the most damaging agricultural pests globally and can be used to investigate this multitrophic network because they host a rich diversity of endosymbionts (17, 18) and share a long co-evolutionary history with some of them dating back approximately 160–280 million years (19). Almost all aphids carry the obligate symbiont *Buchnera aphidicola*, which can provide essential amino acids that aphids cannot obtain from the phloem of plants (5). Some aphids also carry facultative symbionts that exhibit diverse and environmentally dependent effects on hosts (20) and life-history characteristics such as reproduction (21) and dispersal (22) although impacts on host plant use remain unclear (23). Aphids typically feed on a range of host plant species which include crops that are damaged directly or indirectly through virus transmission (24), leading to substantial economic losses (24–26). Their high reproductive potential, capacity to transmit many economically important plant viruses, and widespread resistance to insecticides further exacerbate their impact (24, 27). Additionally, aphids can reproduce asexually and develop winged progeny, enabling them to relocate to new host plants and disperse widely (28).

Recent studies indicate that endosymbionts may influence aphid–plant–virus interactions. In *Acyrtosiphon pisum*, several endosymbionts may modulate host metabolism and feeding behaviour, thereby influencing virus transmission (29). In *Rhopalosiphum padi*, the transinfected endosymbiont *Regiella insecticola* can interfere with the acquisition and transmission of plant viruses (15). These studies highlight a potential level of complexity of interactions involving endosymbionts beyond direct impacts on host species. For major agricultural pests, it is particularly important to understand how such interactions ultimately shape pest population dynamics and impact crop yield, particularly if there are opportunities to deliberately introduce novel endosymbionts to influence these interactions (30).

Here, we explore endosymbiont–insect–plant interactions following the interspecific transinfection of two endosymbionts, *Rickettsiella viridis* and *Regiella insecticola*, into a global pest of cereal crops, the Russian wheat aphid (*Diuraphis noxia* (Mordwilko)). This follows the successful transinfection of *Rickettsiella* from its natural pea aphid host (*Acyrtosiphon pisum*) into the green peach aphid (*Myzus persicae*), where it imposed substantial fitness costs while spreading stably across different host plants through multiple routes (31–33), and the transinfection of *Regiella* from *M. persicae* into its novel aphid host, *R. padi*, with impacts on the transmission of barley yellow dwarf virus (15). While many cross-species aphid transinfections may be unsuccessful or rapidly lost, others can be completely stable (30).

We focused on *D. noxia* due to its importance as an agricultural pest and because native (non-endosymbiotic) bacteria proteins present in *D. noxia* saliva have been linked to aphid feeding damage and virulence to wheat (34). Unlike many other aphid species, *D. noxia* does not transmit plant viruses (35, 36) and rarely carries facultative endosymbionts in the wild (17, 34, 37). *D. noxia* is an invasive pest that has caused major yield losses in wheat and barley, well documented in the USA since its introduction in 1986 (38). Although biotype-specific resistant wheat cultivars can provide temporary control, the continued evolution of *D. noxia* biotypes frequently overcomes host-plant resistance (39, 40). We evaluated the effects of *Rickettsiella* and *Regiella* on aphid fitness, population growth, alate production, plant feeding damage and plant immune responses (SA and JA levels), as well as endosymbiont spread within populations. By integrating these factors, we provide a framework to understand how novel microbial introductions influence endosymbiont–insect–plant interactions, with potential applications in pest management.

Results

Rickettsiella and *Regiella* transinfections

We successfully transinfected *Rickettsiella* from the donor *A. pisum* to *D. noxia* via hemolymph microinjection. Of the fifty aphids injected, twelve survived and six of these produced nymphs that tested positive for *Rickettsiella*; one of these lines transmitted *Rickettsiella* to its offspring. Although *A. pisum* carried both *Serratia symbiotica* and *Rickettsiella*, *Serratia* was not detected in *D. noxia* at G0, whereas *Rickettsiella* was stably maintained at a frequency of 100% for over eighty generations following selection. Similarly, *Regiella* was successfully transinfected from the donor *M. persicae* to *D. noxia*. At G0, eleven of thirty injected aphids tested positive, and three lines exhibited stable vertical transmission to their offspring. These lines were combined at G4, after which *Regiella* persisted at a frequency of 100% for over fifty generations.

Strain-dependent differences in feeding damage to wheat and barley

Rickettsiella and *Regiella* had significant effects on the severity of feeding damage by their host aphids on wheat and barley. On wheat, the *Rickettsiella* strain produced the greatest level of damage, wild type aphids had intermediate effects, and the *Regiella* strain produced the least plant damage (Figure 1A). A repeated-measures ANOVA from Week 2 to Week 3.5 showed a significant main effect of strain on almost all the plant traits, including tiller number ($F_{2,27} = 6.280$, $p = 0.006$, Figure 1B), leaf number ($F_{2,27} = 3.362$, $p = 0.050$, Figure 1C), leaf area ($F_{2,27} = 7.794$, $p = 0.002$, Figure 1D), and overall plant damage (F

$2,27 = 9.099$, $p < 0.001$, Figure 1G), with a weaker and non-significant effect on chlorotic streaking ($F_{2,27} = 3.181$, $p = 0.067$, Figure 1E). All traits exhibited strong effects of time (all $p < 0.001$), reflecting rapid progression of feeding damage across the 3.5 week assessment period. Significant strain $\times$ time interactions were detected for aphid infestation level ($F_{4,54} = 3.476$, $p = 0.007$, Figure 1F) and leaf area ($F_{4,54} = 3.476$, $p = 0.015$, Figure 1D), indicating that the effects of aphid strains changed differently across sampling weeks.

Bonferroni-adjusted pairwise comparisons showed that the *Rickettsiella* strain caused more severe plant damage and supported larger aphid populations than the *Regiella* aphid strain on wheat. Specifically, plants infested with *Rickettsiella* aphids had fewer leaves (Week 3: $p = 0.032$; Week 3.5: $p = 0.028$, Figure 1C), reduced leaf area (Week 2.5: $p = 0.006$; Week 3: $p = 0.002$, Figure 1D), higher aphid numbers (Week 2: $p = 0.004$; Week 2.5: $p < 0.001$; Week 3.5: $p = 0.020$, Figure 1F), and greater overall plant damage (Week 2.5: $p < 0.001$; Week 3.5: $p = 0.022$, Figure 1G) compared with plants infested with *Regiella* aphids. Plants infested with the wild type strain also differed from those infested with *Regiella* aphids, with reduced leaf area (Week 3.5: $p = 0.031$, Figure 1D), greater chlorotic streaking (Week 2.5: $p = 0.031$, Figure 1E), higher aphid numbers (Week 2: $p = 0.031$; Week 2.5: $p = 0.002$, Figure 1F), and greater overall plant damage (Week 2.5: $p < 0.001$, Figure 1G).

On barley, feeding damage patterns were similar to those on wheat, although fewer assessments were significantly different between aphid strains. A repeated-measures ANOVA from Week 2 to Week 3 showed a significant main effect of strain on chlorotic streaking ($F_{2,24} = 5.016$, $p = 0.015$, Figure S1E) and overall plant damage ($F_{2,24} = 4.142$, $p = 0.028$, Figure S1G). As on wheat, the *Rickettsiella* strain produced the greatest level of damage, wild type aphids had intermediate effects, and the *Regiella* strain produced the least damage to barley (Figure S1A). All plant traits showed a strong effect of time (all $p < 0.001$), reflecting an increase in feeding damage across the 3 week assessment period. However, strain $\times$ time interactions were not significant (all $p > 0.095$), indicating similar trends in damage over time across strains. Bonferroni-adjusted pairwise comparisons showed greater chlorotic streaking (Week 2: $p = 0.019$, Figure S1E) and overall plant damage (Week 2: $p = 0.034$, Figure S1G) in plants infested with *Rickettsiella* aphids than with *Regiella* aphids. At Week 3, *Regiella* aphids again tended to produce lower chlorotic streaking ($p = 0.055$) and overall plant damage ($p = 0.056$), although these differences were not statistically significant between strains.

In the barley experiment, we also measured the developmental stages of each aphid strain at Week 3. The *Rickettsiella* aphids had a lower alate frequency than the wild type and *Regiella* aphids (one-way ANOVA: $p < 0.001$, Figure S1I), although total adult numbers and total nymph numbers did not differ among strains (all $p > 0.105$, Figures S1H & S1J).

No effects of *Rickettsiella* and *Regiella* on plant defense responses

Using all GC-MS components, PCA's clearly distinguished metabolic profiles of aphid-feeding areas from non-feeding areas (Figure 2B). However, within either area, no clear separation among aphid strains was observed (Figures 2C & 2D), indicating broadly similar responses to the different aphid strains.

Levels of JA, JA-Ile, and SA did not differ significantly among treatments in either feeding areas (Figure S2A-C) or non-feeding areas (Figure S2D-F), indicating that *Rickettsiella* and *Regiella* did not induce notable differences in defense responses through the JA and SA pathways.

Heatmap analysis showed largely consistent responses among aphid strains in both feeding areas (Figure S3A) and non-feeding areas (Figure S3B), with the exception of four metabolites (Figure S4). In feeding areas, valine (Figure S4A) increased by an average of 1.4-fold, while 3-phenyllactic acid (Figure S4B) decreased by an average of 3.3-fold in plants exposed to *Rickettsiella* aphids compared with plants exposed to wild type aphids, suggesting possible alterations in amino acid metabolism and reduction in antimicrobial or defense-related metabolites. In non-feeding areas, urea (Figure S4C) decreased by an average of 5.3-fold, while pantothenic acid (Figure S4D)

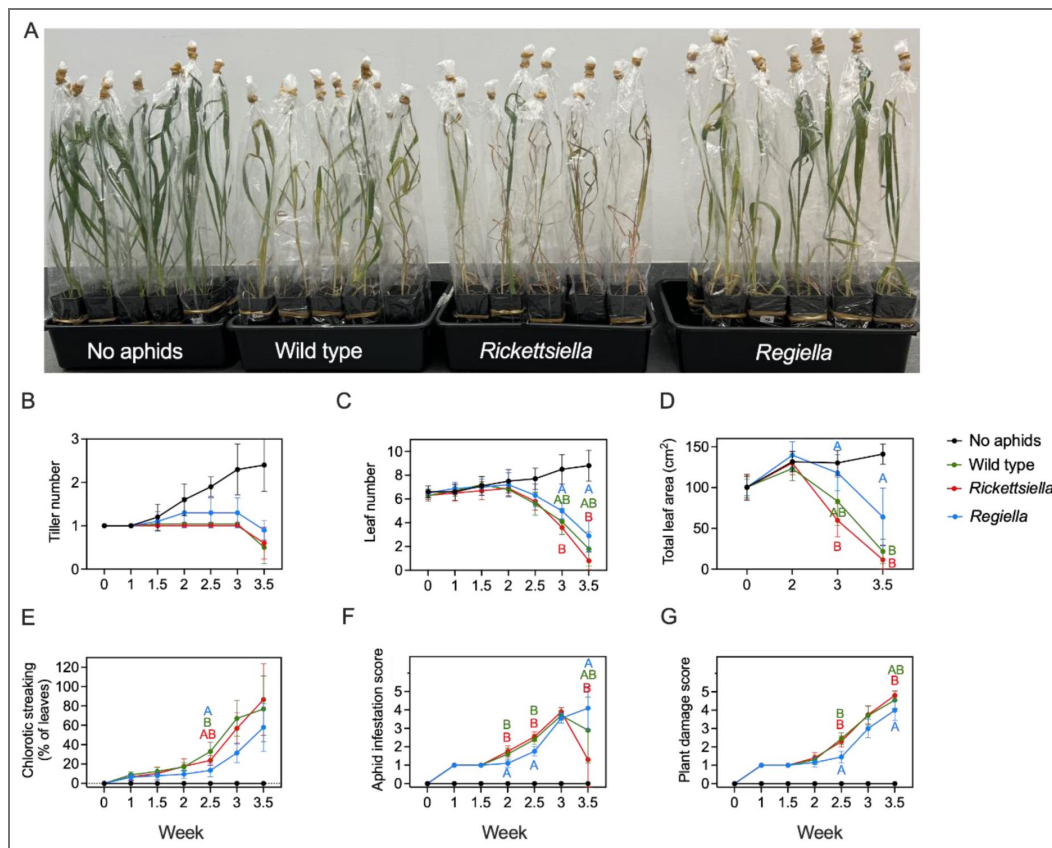


Figure 1. Aphid feeding damage to wheat plants when infested with *Rickettsiella*, *Regiella* or wild type *D. noxia*.

Five age-matched *D. noxia* were placed on individual wheat plants. Wheat plants without aphids were included for comparison. (A) Aphid feeding damage to wheat plants when infested with *Rickettsiella*, *Regiella*, wild type *D. noxia* or when lacking aphids at Week 3.5. Each treatment had 10 replicates and measured traits were (B) tiller number, (C) leaf number, (D) total leaf area, (E) chlorotic streaking, as well as an (F) aphid infestation score and (G) overall plant damage score. Different letters represent significant pairwise differences between treatments by Bonferroni-adjusted tests. Dots represent means across all replicate wheat plants, and error bars indicate 95% confidence intervals.

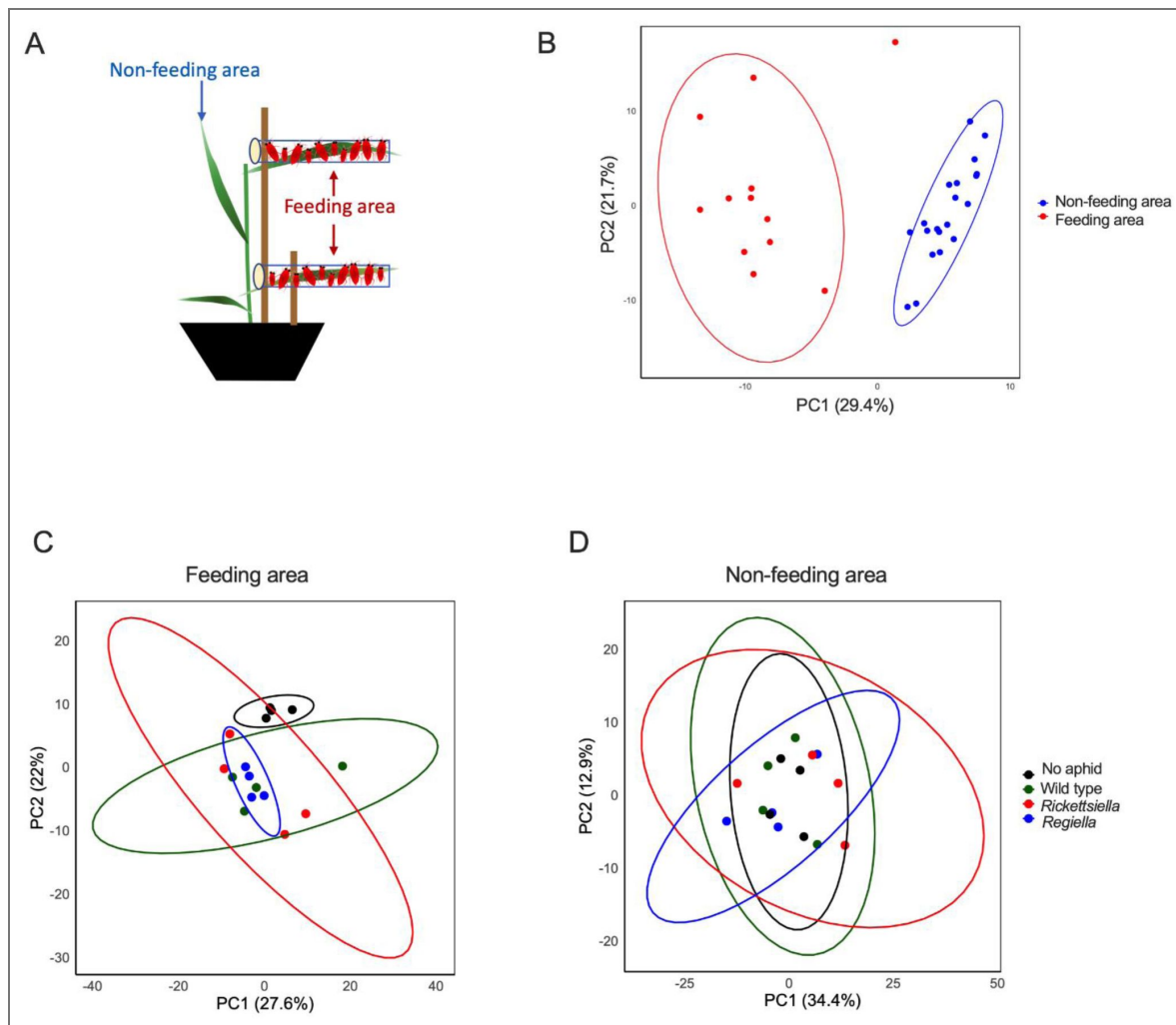


Figure 2. Principal Component Analysis of all GC-MS components showing metabolic profiles:

(A) Schematic showing the feeding and non-feeding areas the wheat plants used in this experiment. Sixty age-mixed aphids were divided into two plastic vials. The top and bottom leaves of each plant were inserted into a vial and a sponge plug was placed into the vial openings and left for 7 days. Control plants were treated in same manner, except no aphids were added to the vials. After a 7-day infestation period, all aphids were removed and the plants remained for an additional 7 days before phytohormone analysis was undertaken. (B) Profiles of aphid feeding areas versus non-feeding areas, (C) profiles of the three aphid strains and no-aphid control on the feeding areas, and (D) profiles of the three aphid strains and no-aphid control on the non-feeding areas. Dots show data from individual replicate plants ($n = 4$ per aphid strain), and ovals show 95% confidence regions. For (B), data from areas with no aphids were included as non-feeding areas. For (C) and (D), the same feeding areas with no aphids were included as controls for comparison with the different aphid feeding treatments.

increased by an average of 1.5-fold in plants exposed to *Regiella* aphids compared to plants exposed to wild type aphids, potentially indicating altered nitrogen metabolism and enhanced energy and secondary metabolite synthesis caused by *Regiella* aphid feeding.

No effects of *Rickettsiella* and *Regiella* on aphid life history traits

We measured the effects of *Rickettsiella* and *Regiella* on life history traits of their host aphids at two temperatures (19°C and 25°C), as well as body color and body shape/size (Figures S5 & S6). For *Rickettsiella*, development time differed between strains (Kruskal-Wallis Test: $H_{1,62} = 4.405$, $p = 0.036$, Figure S5A), with *Rickettsiella* aphids developing faster than wild type aphids at 25°C. However, lifetime fecundity (GLM: $F_{1,118} = 1.321$, $p = 0.253$, Figure S5B) and longevity (Cox regression: $\chi^2 = 0.153$, d.f. = 1, $p = 0.696$, Figure S5C) did not differ between *Rickettsiella* and wild type aphids at either temperature, and there was no significant effect on development time at 19°C. Temperature significantly affected development time (Kruskal-Wallis test: $H_{1,122} = 86.061$, $p < 0.001$) and longevity (Cox regression: $\chi^2 = 10.635$, d.f. = 1, $p = 0.001$) but not lifetime fecundity (GLM: $F_{1,118} = 0.033$, $p = 0.857$). Unlike in other aphid species (15, 31, 41), *Rickettsiella* had no effect on body color in *D. noxia* (MANOVA across all color components: $p = 0.618$; t-test: Hue: $p = 0.795$, Figure S5D; Saturation: $p = 0.409$, Figure S5E; Lightness: $p = 0.603$, Figure S5F). Body shape of apterous adults (expressed as the ratio of body length/body width) was also not influenced by *Rickettsiella* infection (t-test: $p = 0.115$, Figure S5G).

At 19°C, we measured *Buchnera* and *Rickettsiella* densities, and found no effect of *Rickettsiella* on *Buchnera* density (t-test: $p = 0.914$, Figure S5H), suggesting the addition of a novel *Rickettsiella* endosymbiont did not perturb the obligate endosymbiont *Buchnera*. All *Rickettsiella* aphids we screened were positive for *Rickettsiella*, with a median density equivalent to the host control gene, and all wild type aphids screened were negative (Figure S5I).

For *Regiella*, there were no effects on development time (Kruskal-Wallis Test: $H_{1,148} = 0.449$, $p = 0.503$, Figure S6A), lifetime fecundity (GLM: $F_{1,113} = 5.266$, $p = 0.919$, Figure S6B), longevity (Cox regression: $\chi^2 = 1.719$, d.f. = 1, $p = 0.190$, Figure S6C), or the body length of apterous adults ($F_{1,120} = 2.063$, $p = 0.154$, Figure S6G) relative to wild type aphids at both 19°C and 25°C. *Regiella* affected body color components except for hue (GLM: Hue: $F_{1,119} = 0.658$, $p = 0.544$, Figure S6D; Saturation: $F_{1,119} = 941.134$, $p < 0.001$, Figure S6E; Lightness: $F_{1,119} = 29.579$, $p < 0.001$, Figure S6F), with *Regiella* aphids being lighter in color and less saturated than wild type aphids. Temperature significantly affected development time (Kruskal-Wallis test: $H_{1,148} = 103.232$, $p < 0.001$), longevity (Cox regression: $\chi^2 = 6.904$, d.f. = 1, $p = 0.009$), and lifetime fecundity (GLM: $F_{1,113} = 12.494$, $p < 0.001$), but did not affect body color traits except hue ($F_{1,119} = 19.364$, $p < 0.001$). No significant strain $\times$ temperature interactions were detected for any measured trait (all $p > 0.085$).

At 19°C, *Regiella* did not significantly affect *Buchnera* density (t-test, $p = 0.075$; Figure S6H), suggesting the addition of a novel *Regiella* endosymbiont did not perturb the obligate endosymbiont *Buchnera*. All *Regiella* aphids we screened were positive for *Regiella*, with a median density equivalent to the host control gene, and all wild type aphids screened were negative (Figure S6I).

Frequency of *Rickettsiella* and *Regiella* in mixed infection cages linked to temperature

We established cages at both 19°C and 25°C containing mixed populations of wild type aphids and aphid strains carrying either *Rickettsiella* or *Regiella*, to investigate population dynamics over time (Figures S7 & S8). The proportion of aphids carrying *Rickettsiella* or *Regiella* increased rapidly. *Rickettsiella* reached >75% frequency in most replicate cages by Week 3 at 19°C (Figure S7B) and by Week 2 at 25°C (Figure S7C). *Regiella* reached 100% frequency at both temperatures by Week 12 (Figures S8B and S8C). At 25°C, *Rickettsiella* maintained a high prevalence through Week 12 (Figure S7C), whereas at 19°C its prevalence declined between Weeks 9 and 12 (Figure S7B). By contrast, *Regiella* persisted at a higher frequency at 19°C for 30 weeks (Figures S8B) but at 25°C its frequency varied among replicate cages at Weeks 18 and 24 (Figures S8C).

Horizontal transmission is expected to initially generate aphids with low endosymbiont densities, which may then increase and be vertically transmitted to subsequent generations (31). For *Rickettsiella*, aphids with low endosymbiont densities were common (Figure S7F and S7G) but occurred at relatively lower frequencies at 25°C (Figure S7G). High-density infections generally remained stable over time for *Rickettsiella* at 25°C (Figure S7E) and for *Regiella* at 19°C (Figure S8D), whereas high *Regiella* densities declined at 25°C in some replicate cages from Week 12 (Figures S8E and S8G). The persistence of high-density infections is consistent with minor fitness costs associated with both infections, although any minor fitness disadvantage in infected individuals could also be offset by conversion from low-density infections.

Different effects of *Rickettsiella* and *Regiella* on aphid population growth and alate production on whole plants

We investigated the effects of *Rickettsiella* and *Regiella* on aphid population growth and alate production on whole wheat plants. In the *Rickettsiella* experiment, plant numbers were limited (three plants per time point), so analyses focused on Days 21 and 25 where any population growth effects were expected to have built up. There was no effect of strain on total adult numbers (alate + apterous adults) at these two time-points ($F_{1,8} = 2.159$, $p = 0.180$; Figure 3B), and there was no time $\times$ strain interaction ($F_{1,8} = 0.468$, $p = 0.513$). However, *Rickettsiella* significantly reduced alate frequency from Day 14 to Day 25 (GLM: $F_{1,12} = 26.434$, $p < 0.001$, Figure 3C), and the strain $\times$ time interaction was also significant (GLM: $F_{2,12} = 7.830$, $p = 0.007$). The frequency of alates decreased as plant damage increased (Figure 3C) but remained significantly lower for the *Rickettsiella* strain at all sampling points. Total nymph numbers were also higher on plants infested with *Rickettsiella* aphids from Day 21 to Day 25 than on plants infested with wild type aphids (GLM: $F_{1,8} = 13.646$, $p = 0.006$, Figure 3D), with no strain $\times$ time interaction ($F_{1,8} = 1.070$, $p = 0.331$).

In the *Regiella* experiment, we used a slightly different design whereby aphids were introduced at multiple positions on individual wheat plants (see “Population growth and alate production on whole plants”). Here, we focused on the period after 4 days when numbers were still building up. Adult *Regiella* aphids were significantly fewer than wild type adults on Days 8 and 12 ($F_{1,14} = 14.049$, $p = 0.002$, Figure 4B), but this difference was not evident at Day 16 ($F_{1,19} = 1.045$, $p = 0.320$). The time $\times$ strain interaction for adult numbers from Day 8 to Day 16 was significant ($F_{2,19} = 3.868$, $p = 0.039$, Figure 4B). Aphid strain did not affect alate frequency (Day 8 to 12: $F_{1,14} = 0.040$, $p = 0.844$; Day 16: $F_{1,19} = 1.385$, $p = 0.254$; Figure 4C) or the total nymph number (Day 8 to 12: $F_{1,14} = 4.315$, $p = 0.057$; Day 16: $F_{1,19} = 0.708$, $p = 0.411$; Figure 4D). Time $\times$ strain interactions were also not significant for alate frequency ($F_{2,19} = 0.893$, $p = 0.426$, Figure 4C) or total nymph number ($F_{2,19} = 0.599$, $p = 0.622$, Figure 4D) from Day 8 to Day 16.

Rickettsiella reduces alate production and increases feeding damage to wheat plants in mesocosms

We further tested the effects of *Rickettsiella* on aphid dispersal ability and feeding damage to wheat plants in a mesocosm experiment in which *Rickettsiella* and wild type aphids were released into the same enclosures. Patterns of feeding damage were consistent with our earlier experiments using individual cages (Figure 1), with greater feeding damage over time in the *Rickettsiella* treatment than in the wild type treatment (Figure 5). The clearest differences occurred at Week 3.5 on the aphid-release plants, where plants infested with *Rickettsiella* aphids had fewer leaves (t-test: $p < 0.001$, Figure 5B), reduced total leaf area ($p = 0.050$, Figure 5C), higher aphid infestation scores ($p < 0.001$, Figure 5F), and greater overall plant damage ($p = 0.007$, Figure 5G) than plants infested with wild type aphids. Chlorotic streaking also appeared earlier and was greater in plants infested with *Rickettsiella* aphids, with a significant difference at Week 3 ($p = 0.046$, Figure 5D). Similar results were observed for the repeated measures GLM; wheat plants infested with *Rickettsiella* aphids had fewer leaves (GLM: $F_{1,38} = 11.368$, $p = 0.002$), reduced total leaf area ($F_{1,38} = 14.849$, $p < 0.001$), greater chlorotic streaking before damage levels converged (Weeks 2-3.5: $F_{1,31} = 7.409$, $p = 0.011$) and greater overall plant damage ($F_{1,38} = 12.414$, p

Figure 3. *Rickettsiella* effects on aphid population growth and alate production on whole plants.

(A) Experimental design for *Rickettsiella*. Five age-matched *Rickettsiella* or wild type aphids were placed onto wheat plants (GS14). At Day 14, 21, and 25, aphids from three replicate plants per treatment were removed and the number of alate adults, apterous adults and nymphs was recorded. (B) The number of *Rickettsiella* adults (alate plus apterous adults), (C) the percentage of *Rickettsiella* alates and (D) the number of *Rickettsiella* nymphs were used to characterize aphid population growth and alate production over time. “*” indicates significant differences ($p < 0.05$) between endosymbiont and wild type treatments at each time point, as determined by independent sample t-tests.

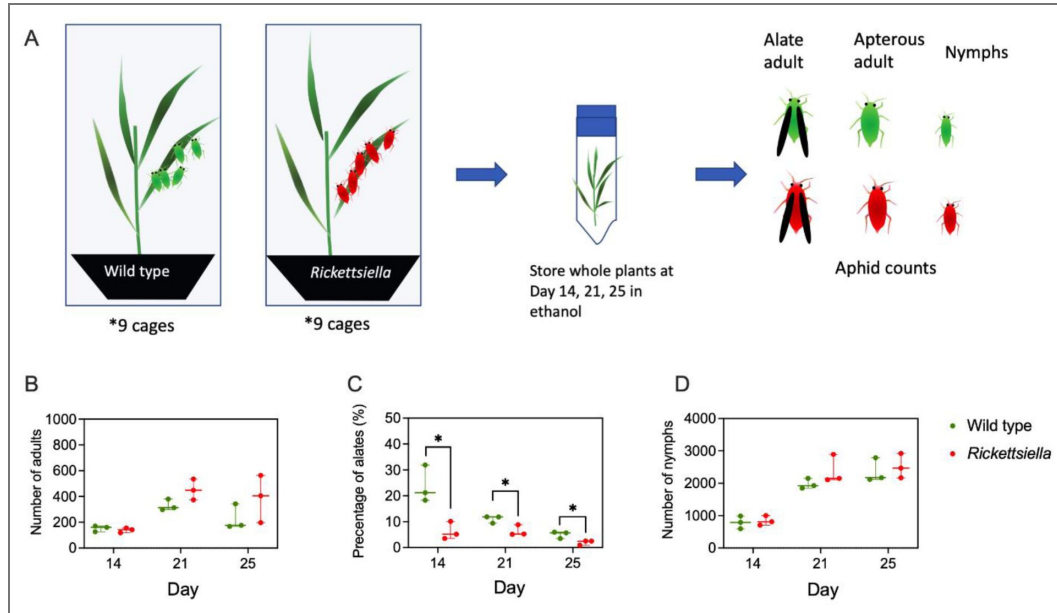
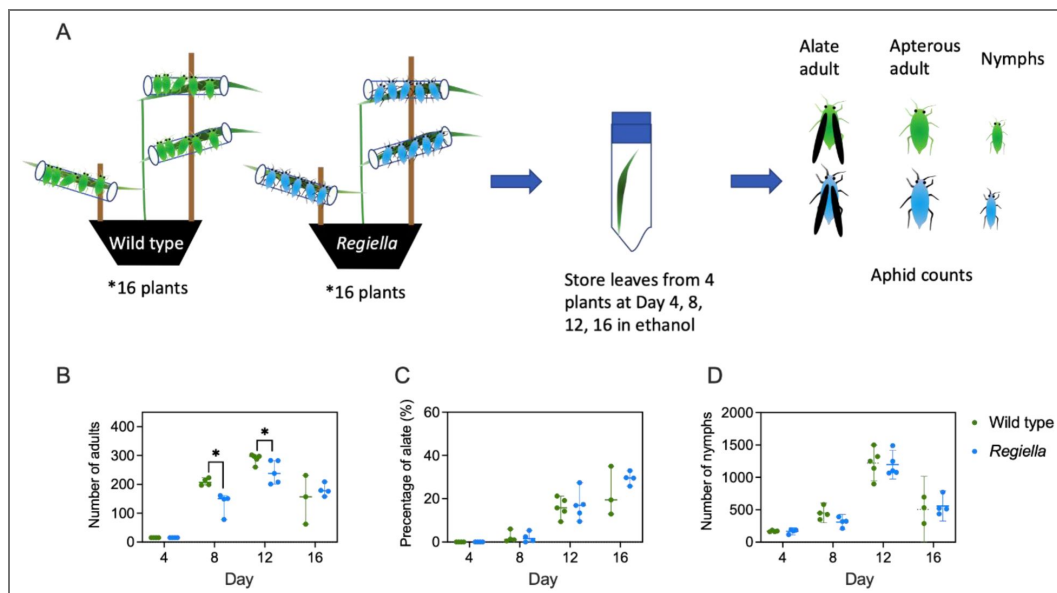


Figure 4. *Regiella* effects on aphid population growth and alate production on whole plants.

(A) Experimental design for *Regiella*. Five age-matched *Regiella* or wild type aphids were placed onto each of the top, middle and bottom leaves of wheat plants (GS14). A plastic vial was placed over each leaf and cotton wool inserted into the vial opening to prevent aphid movement. At Day 4, 8, 12, and 16, aphids from four replicate plants per treatment were removed and the number of alate adults, apterous adults and nymphs was recorded. (B) The number of *Regiella* adults (alate plus apterous adults), (C) the percentage of *Regiella* alates and (D) the number of *Regiella* nymphs were used to characterize aphid population growth and alate production over time. “*” indicates significant differences ($p < 0.05$) between endosymbiont and wild type treatments at each time point, as determined by independent sample t-tests. Development stages of aphids on different wheat leaves are shown in Figure S9.



= 0.001). When a combination of all plant traits was considered in the MANOVAs, feeding damage to wheat plants differed significantly between aphid strains at Week 2 ($p = 0.023$) but differences were marginally non-significant at Week 3 ($p = 0.050$) and Week 4 ($p = 0.070$).

Increased feeding damage from *Rickettsiella* aphids was also evident in the aphid-spread plants, and this effect was most pronounced at Week 4.5. At this time point, there were fewer leaves (t-test: $p = 0.003$, Figure 5B), and greater overall plant damage ($p = 0.001$, Figure 5G) in the *Rickettsiella* treatment compared with the wild type treatment. Besides, *Rickettsiella* treatment also had a higher aphid infestation ($p = 0.001$, Figure 5F). Total leaf area was also significantly lower in the *Rickettsiella* treatment than in the wild type treatment at Week 4 ($p = 0.020$, Figure 5C). A similar pattern was observed for the repeated measures GLM of the aphid-spread plants; plants infested with *Rickettsiella* aphids had reduced leaf area ($F_{1,38} = 4.432$, $p = 0.042$) and greater overall plant damage ($F_{1,38} = 6.009$, $p = 0.019$).

If *Rickettsiella* and wild type aphids exhibited similar population growth and dispersed equally, we would expect a *Rickettsiella* frequency of 50% among aphids sampled from the middle plants. However, at both Weeks 2 and 3, the proportion of alates in the middle tray that tested positive for *Rickettsiella* was significantly below 50% (paired t-test: $p = 0.002$, Figure 5H). In contrast, no significant deviation from 50% was observed for apterous aphids collected from the middle tray ($p = 0.922$). As expected, the numbers of alate (Figure 5I) and apterous adults (Figure 5J) in the middle trays increased over time.

Discussion

Despite the wide-ranging effects of facultative endosymbionts on aphid phenotypes, relatively few studies have considered endosymbiont–aphid–plant interactions, although enhanced plant immune responses have been reported in some systems (16, 42). Here, we show that transinfections of *Rickettsiella* and *Regiella* in *D. noxia* produced opposing effects on plant damage. *Rickettsiella* increased aphid feeding damage, whereas *Regiella* decreased it in both wheat and barley (Figures 1 and S1, Table S2). These effects were evident across multiple plant traits, and were particularly clear for chlorotic streaking, which is an important effect reflecting salivary toxins secreted during *D. noxia* feeding (43). However, we did not detect any differences in plant immune responses through the JA or SA pathways, suggesting that the endosymbionts did not reprogram canonical defense signalling in wheat under our experimental conditions.

Despite this, our metabolomic analyses suggest that endosymbionts in *D. noxia* may influence some other aspects of wheat metabolism in a spatially structured way. Within aphid-feeding areas, wheat exposed to *Rickettsiella* aphids showed increased valine and decreased 3-phenyllactic acid; valine accumulation can reflect a plant stress response to herbivory (44–46), while 3-phenyllactic acid has been associated with antimicrobial and defense-related activity (47). In the non-feeding areas, wheat exposed to *Regiella* aphids showed reduced urea and increased pantothenic acid. Decreased urea may reflect shifts in nitrogen allocation or metabolism (48), while increased pantothenic acid may support fatty acid and secondary metabolite synthesis (49). These results are worth following up with additional work and support the notion that phloem-feeding insects can induce changes in plant metabolites in response to herbivory (50, 51).

Apart from inducing metabolic changes, endosymbiont effects on plant damage may reflect population-level effects rather than (or in addition to) direct plant-endosymbiont interactions. In single plant experiments, we detected few fitness effects attributable to endosymbionts, but our population growth results showed *D. noxia* carrying *Rickettsiella* led to a greater population size, whereas *Regiella* aphids showed a reduction in size. These demographic effects could contribute to differences in feeding pressure and symptom development. The direction of the *Rickettsiella* effects in *D. noxia* differs from patterns reported in transinfected *M. persicae* (31, 32), underscoring that fitness consequences of facultative symbionts are host-specific.

Both endosymbionts persisted and spread rapidly in mixed cages, with dynamics partly influenced by temperature. *Rickettsiella* frequency declined late in the 19°C cages, whereas *Regiella* ultimately reached fixation at both temperatures but showed greater among-replicate variability at 25°C later

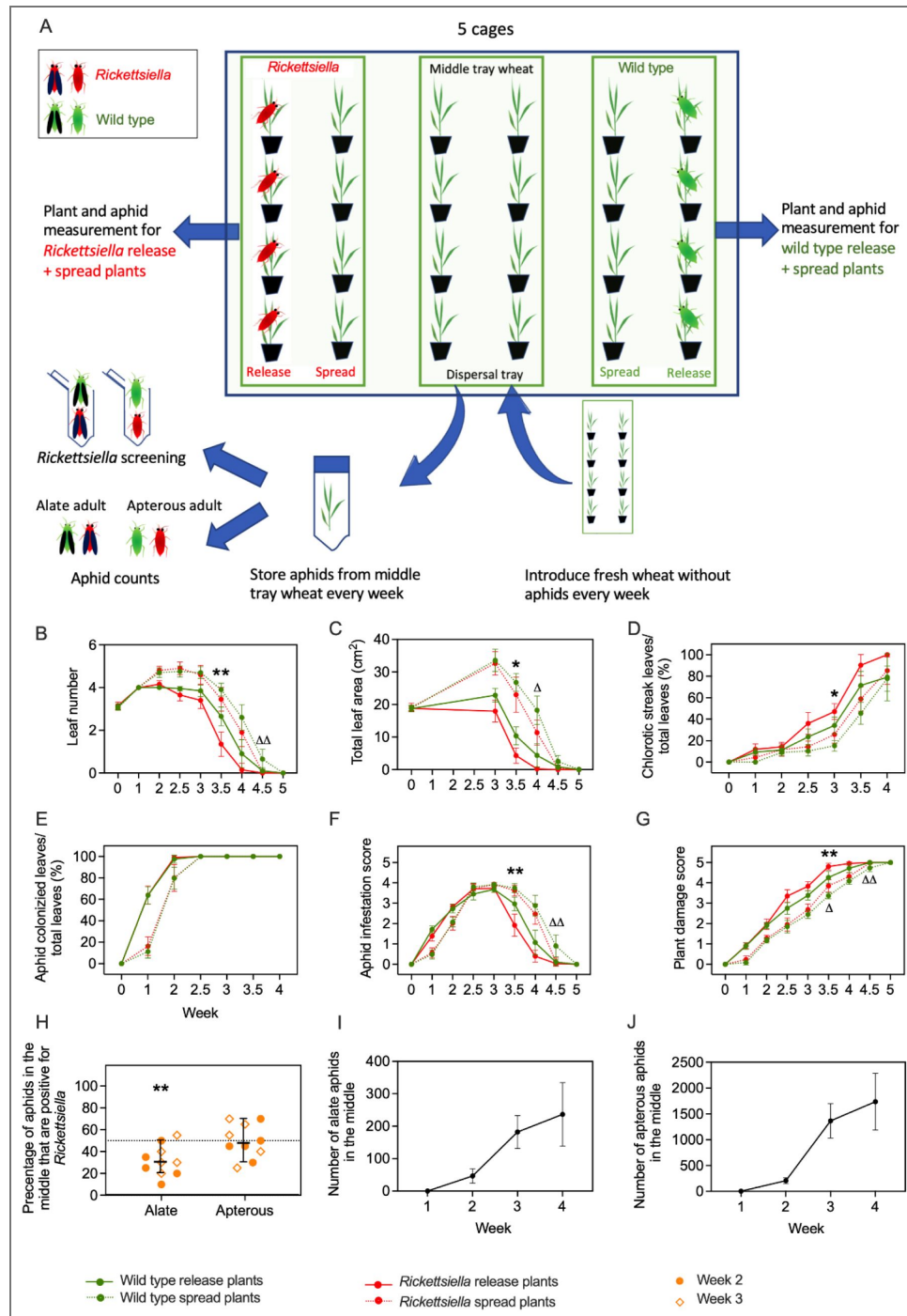


Figure 5. *Rickettsiella* effects on aphid dispersal and plant feeding damage to wheat plants in mesocosms.

(A) Experimental design. Populations were initiated with 5 *Rickettsiella* or wild type aphids on wheat plants ('aphid-release wheat') while adjacent plants ('aphid-spread wheat') were monitored. All plants were measured for (B) leaf number, (C) total leaf area, (D) chlorotic streaking, (E) aphid colonization rate, as well as (F) an aphid infestation score and (G) overall plant damage score. "**" and "***" represent significant differences at $p < 0.05$ and $p < 0.01$, respectively when comparing *Rickettsiella* and wild type aphids on aphid-release wheat. "Δ" and "ΔΔ" represent significant differences at $p < 0.05$ and $p < 0.01$, respectively when comparing *Rickettsiella* and wild type aphids on aphid-spread wheat. Aphids collected from the middle tray ("dispersal") were counted and screened to determine (H) the percentage of alate and apterous adults in the middle that were positive for *Rickettsiella*, (I) the number of alate adults, and (J) the number of apterous adults. Symbols in (H) represent the percentage of 20 alate and 20 apterous adults positive for *Rickettsiella* at Weeks 2 and 3. "***" represents a significant difference at $p < 0.01$ as determined from a paired t-test.

in the experiment. These outcomes are potentially shaped by both vertical transmission and horizontal transmission through plant tissue and/or transfer via aphid contact or honeydew. Similar patterns of context-dependent symbiont effects have been reported in other aphid species (52) and may be relevant to variability in aphid endosymbiont frequencies under field conditions where additional factors such as parasitism interactions can be important (53–55). Although we did not directly document conversion of low endosymbiont titre to high titre offspring as previously demonstrated for *Rickettsiella* in *M. persicae* (31), we observed a substantial increase in the incidence of high-titre infections of both endosymbionts following periods of increase in the total incidence of infections, which suggests that this conversion may be ongoing. However serial bottlenecks during transfers likely generated a high level of variability among replicates.

A noteworthy result is that *Rickettsiella* reduced alate production in *D. noxia*, whereas *Regiella* did not. Wing polymorphism is an important adaptive response that facilitates aphid dispersal in response to crowding, host quality, and other environmental cues (56). Previous studies have shown that *Serratia* can promote wing development in *A. pisum* that enhances its dispersal (22) and that suppression of *Buchnera* through antibiotic treatment can reduce alate production in *Sitobion avenae* (57). We have also found that transinfection of *Rickettsiella* into *R. padi* reduced both host fitness and alate production, further supporting an effect of this endosymbiont in dispersal-related traits across aphid hosts (58). The suppression of wing development and alate dispersal by *Rickettsiella* in *D. noxia* across experiments in wheat and barley was apparent even when total adult numbers were high, indicating that the effect persists irrespective of crowding. In future work, it may be possible to directly measure the effects of *Rickettsiella* on wing formation as done in other studies (e.g. 22). Given wing formation is highly sensitive to environmental conditions (59), it would also be interesting to complete multiple-generation experiments in large arenas at different temperatures and using plants experiencing different stress levels.

It would be worthwhile undertaking further work aimed at understanding of the mechanisms underlying the symbiont-associated differences in plant damage. Aphid feeding behavior could be examined using electrical penetration graph (EPG) assays to test whether symbionts alter probing dynamics, phloem access, or toxic components in aphid saliva. Recent work has shown that endosymbionts can alter probing and feeding behaviour (60, 61) and the composition of salivary proteins (62) in aphids. Virulent biotypes of *D. noxia* are known to produce more additional energy than less virulent biotypes under stressful conditions based on measures of the NAD⁺/NADH ratio (63), and such differences could also be impacted by endosymbionts (64). Luna et al. (2018) (65) identified bacterial proteins in *D. noxia* saliva that facilitates virulence to wheat. Apart from bacteria, recent studies have also showed non-coding RNA (66) and epigenetic effects (66) that alter virulence to host plants. Comparing salivary toxins and regulatory pathways among *Rickettsiella*, wild type and *Regiella* aphids could therefore help identify how symbionts influence damage-related traits, and potentially contribute to understanding how *D. noxia* develops new virulent populations despite limited genetic diversity (67).

The transinfected strains could be useful in aphid management depending on the ecology of the pest populations. If *Regiella* reduces plant damage by suppressing *D. noxia* population growth, it could potentially lessen crop impact once established in populations. Conversely, *Rickettsiella* establishment may limit aphid spread. These potential benefits need to be weighed against risks which will likely depend on the cropping situation. In related work, we have found that enhanced plant damage from *Rickettsiella* extends beyond wheat and barley to summer ‘green-bridge’ hosts such as barley grass and brome grass (68, 69), which could help reduce *D. noxia* populations prior to the cropping season. The combined effects of *Rickettsiella* on dispersal and green bridge hosts could offset any increases in crop damage.

Implementation of endosymbiont-based programs will also depend on clarifying other risks. Current data suggest that *Rickettsiella* transinfections have little impact on pesticide tolerance in *D. noxia* and other aphids (70), an important risk dimension given that chemicals remain the major way *D. noxia* are managed in agricultural fields in Australia (69). The experimental evaluations also need to be extended to a broader range of wheat varieties, particularly resistant lines, although this is not currently feasible locally because only *D. noxia*-susceptible varieties are

available in Australia. It would also be worthwhile exploring effects in other *D. noxia* clones given that symbiont effects can vary with aphid genotype (55, 71) although only a single lineage of *D. noxia* has so far been identified in Australia (Owen Holland et al., unpublished data) which likely reflects the recent incursion of this species from overseas (72). Nevertheless, the current results suggest that transinfections can affect *D. noxia* behaviour and plant interactions in interesting ways that might be useful from a control perspective.

Materials and methods

Aphid strains and maintenance

Aphids were cultured using a cup culturing method outlined in Yu, et al. (15). Plant material was inserted into cups with a reservoir of water to support the plant material and aphid population. The host plant for *D. noxia* was wheat (*Triticum aestivum* cv. Trojan), for *M. persicae* it was bok choy (*Brassica rapa* subsp. chinensis var. baby bok), and for *A. pisum* it was lucerne (*Medicago sativa*, cv. Sequel). Aphids were moved to new cups with fresh leaf material twice a week and maintained in temperature-controlled cabinets at 19 (± 1)°C with a 16:8h L:D photoperiod. All plant material was grown in insect-proof BugDorm cages (93 x 47.5 x 47.5cm, mesh 160 µm aperture, Australian Entomological Supplies, Australia) within a shade-house supported with plant growth lights (40W Grow Saber LED 6500K, 1200mm length) set to a 16:8h L:D photoperiod. Wheat and bok choy plants were grown for ~ 6 weeks before use, while lucerne plants were ~ 4.5 weeks old before use. For our experiments, wheat plants (~ 5 weeks old) and barley plants (*Hordeum vulgare* var. La Trobe ~ 5 weeks old) were grown under the same conditions.

For donor species that carried the facultative endosymbionts, a single clone of *A. pisum* was collected from Tintinara, South Australia, Australia (GPS: -35.95S, 140.14E), which carried both *Serratia symbiotica* and *Rickettsiella viridis*, and a single *M. persicae* clone was collected in 2023 from Burnley, Victoria, Australia (GPS: -35.83S, 145.02E), which carried *Regiella insecticola*. For the recipient *D. noxia*, we used a single clone collected from Horsham, Victoria, Australia (GPS: -36.72S, 142.18E). This clone has previously been found to lack naturally occurring facultative endosymbionts (17). We generated a *D. noxia* strain carrying *Rickettsiella* derived from *A. pisum* and another *D. noxia* strain carrying *Regiella* transinfected from *M. persicae* (see “Endosymbiont transinfection through microinjection”). The same clone of *D. noxia* without facultative endosymbionts (wild type) was used as a control in all experiments.

Endosymbiont transinfection through microinjection

We introduced *Rickettsiella* from *A. pisum* and *Regiella* from *M. persicae* into apterous adult *D. noxia* through hemolymph microinjection (31, 73). We injected 50 *D. noxia* with hemolymph from *A. pisum* and 30 *D. noxia* from *M. persicae*, and then followed a selection step as described in Gu, et al. (31) for three generations. Aphids testing positive for infection were then maintained in groups from G4. Endosymbiont status was routinely screened as well as immediately before experiments commenced through quantitative PCR (qPCR) (see “Endosymbiont detection and quantification”).

Endosymbiont detection and quantification

qPCR assays were used to confirm the presence or absence of *Rickettsiella*, *Regiella* and/or *Buchnera* and to measure their densities relative to a host gene following established methods (74). In the experiments assessing life history traits and population dynamics, aphids with a *Rickettsiella* and/ or *Regiella* Cp value ≤ 30 and Tm values within the range of positive controls (87.5–88.3) were considered positive with high density, while aphids with a Cp value >30 were considered positive but with low density, and negative if Cp values were absent and Tm values were not within the range of positive controls. In the mesocosm experiment assessing aphid dispersal and feeding damage, aphids with a *Rickettsiella* Cp value >30 were considered *Rickettsiella* negative due to the potential for horizontally transmitted *Rickettsiella* resulting in wild type aphids testing positive with low densities (Figure S10).

Feeding damage to wheat and barley

D. noxia causes yield loss through direct feeding and leaf chlorosis by injecting toxins into the plant (43). We examined the effect of *Rickettsiella* and *Regiella* on plant damage severity to wheat and barley plants. Wheat and barley were used at Zadok's growth stage GS14 (~ 5 weeks old). Five age-matched apterous adults (10 d old) were placed onto the second true leaf of each plant.

For wheat, individual plants were enclosed in perforated bread-stick plastic bags, secured with rubber bands and placed in randomized positions in a temperature-controlled cabinet at 25°C ($\pm$ 1°C) with a 16:8 h L:D photoperiod under plant growth lights. Ten replicates were established per treatment: *Rickettsiella*, *Regiella*, wild type, and no-aphid controls (40 plants in total). For barley, individual plants were placed in BugDorm cages (30 x 30 x 62 cm, mesh 160 μ m aperture, Australian Entomological Supplies, Australia) and then placed in randomized positions in a temperature-controlled cabinet at 25°C ($\pm$ 1°C) with a 16:8h L:D photoperiod under plant growth lights. Eight replicates were established per treatment (32 plants in total). Initial aphid survival was assessed at 24 h and 48 h post-introduction in both experiments.

For each replicate plant, we recorded the number of tillers, the number of true leaves, leaf area (summed across leaves as width*length*0.75 (75)), and the percentage of leaves with chlorotic streaking. We also measured the level of aphid infestation on each plant and scored an overall plant damage score (see Table S1 for details). These assessments were undertaken either once or twice per week. All scores were assessed independently and blindly by two observers and then averaged to obtain an overall score for each plant at each time point. If the scores assigned by the two observers differed by more than one unit for a given plant, a third observer independently assessed the plant, and the average score from all three observers was used for the final score. The experiments were terminated when plants in one of the replicates had an overall plant damage score of 4 or above (Week 3.5 for wheat and Week 3 for barley). By Week 3.5, all replicate wheat plants infested with *Rickettsiella* aphids were nearly dead, with only a few aphids remaining alive. Therefore, aphid morph composition was assessed only in barley at Week 3. For this assessment, we counted the total number of nymphs, alate adults, and apterous adults on each barley plant.

Plant defense responses

To determine the response of plants to aphid feeding with different endosymbionts, sixteen wheat plants were grown for 5 weeks as described above (see "Aphid strains and maintenance"). Four replicate plants were established for each treatment: *Rickettsiella*, *Regiella*, wild type, and no-aphid controls. For each plant, 60 aphids of mixed age (collected at random from wheat plants within eight BugDorm cages used to maintain aphids) were divided into two plastic vials (Top diameter: 25 mm; height: 95 mm, DrosoKing, Australia). The top and bottom leaves of each plant were inserted into a vial and a sponge plug was placed into the vial opening to prevent aphid movement (see Figure 2A 4). Control plants were treated in same manner, except no aphids were added to the vials. All plants were then placed in randomized positions in a temperature-controlled cabinet at 25°C ($\pm$ 1°C) with a 16:8 h L:D photoperiod under plant growth lights. After a 7-day infestation period, all aphids were removed and the plants maintained for an additional 7 days. This time period was chosen based on a pilot experiment indicating that endogenous JA and SA typically display distinct changes during this time. Plant material (~200 mg) was excised from the top and bottom leaves (aphid feeding areas) and the middle leaf (non-feeding area) of each wheat plant was placed in 60 mL centrifuge tubes and immediately frozen in liquid nitrogen. Fifty mg of this material was then cryomilled (3 x 30 sec, 3620 g) in 600 μ L of 3:1 MeOH/water containing 0.28 nmol of $^{13}\text{C}_5$ ^{15}N valine, $^{13}\text{C}_6$ sorbitol, dihydrojasmonic acid and salicylic acid-d6, using a Bertin Technologies Precellys bead mill coupled to a Cryolys cooling unit. 450 μ L of each homogenate was transferred into fresh Eppendorf tubes and centrifuged at 4°C for 10 min at 16,000 g using an Eppendorf centrifuge 5430 R. The resulting supernatants were then transferred into fresh 1.5 mL Eppendorf tubes. An 80 μ L aliquot of each sample was pooled to create the pooled biological quality control (PBQC). Two hundred μ L of each study sample and the PBQC were transferred into HPLC inserts and evaporated at 30°C to complete dryness, using a CHRIST RVC 2-33 CD plus speed vacuum. To limit the amount of moisture present in the insert, 50 μ L 100%

methanol (LCMS grade) was added to each insert and evaporated using a speed vacuum. Dried samples for GCMS analysis were derivatised online using the Shimadzu AOC6000 autosampler robot. Derivatisation was achieved by the addition of 25 μ L methoxyamine hydrochloride (30 mg/mL in pyridine, Merck) followed by shaking at 37°C for 2h. Samples were then derivatised with 25 μ L of N,O-bis (trimethylsilyl)trifluoroacetamide with trimethylchlorosilane (BSTFA with 1% TMCS, Thermo Scientific) for 1h at 37°C. The sample was allowed to equilibrate at room temperature for 1 h before 1 μ L was injected onto the GC column using a hot needle technique. A splitless injection was performed for each sample.

The GC-MS system used comprised of an AOC6000 autosampler, a 2030 Shimadzu gas chromatograph and a TQ8050NX triple quadrupole mass spectrometer (Shimadzu, Japan) with an electron ionisation source (−70eV). The mass spectrometer was tuned according to the manufacturer's recommendations using tris-(perfluorobutyl)-amine (CF43). GC-MS was performed on a 30m Agilent DB-5 column with 0.25mm internal diameter column and 1 μ m film thickness. The injection temperature (inlet) was set at 280°C, the MS transfer line at 280°C and the ion source adjusted to 200°C. Helium was used as the carrier gas at a flow rate of 1 mL/min and argon gas was used in the collision cell to generate the MRM product ion. The analysis of the derivatised samples was performed under the following oven temperature program; 100°C start temperature, hold for 4 minutes, followed by a 10°C/min oven temperature ramp to 320°C with a following final hold for 11 minutes. Samples were analysed in a randomised order, with a pooled biological quality control sample added after every 5 samples. The pooled biological quality control was used to monitor downstream sample stability and analytical reproducibility. Approximately 625 targets were collected using the Shimadzu Smart Metabolite Database, where each target comprised a quantifier MRM along with a qualifier MRM, which covers approximately 420 unique endogenous metabolites and multiple stable isotopically labelled internal standards. Resultant data was processed using Shimadzu LabSolutions Insight software, where peak integrations were visually validated and manually corrected where required. JA and jasmonic acid-isoleucine (JA-Ile), were quantified by comparing the peak area to the spike in dihydrojasmonic acid (not endogenously found) and SA was quantified by comparing the peak area to the spike in salicylic acid-d6 standard. All the other components were normalized by the peak area to the spike in the $^{13}\text{C}_5$ ^{15}N valine.

Aphid life history traits at two temperatures

We measured the effects of *Rickettsiella* and *Regiella* on development time, lifetime fecundity and longevity following the methods described by Gu, *et al.* (31). Two nymphs (< 24 h old) were transferred to a plastic cup (150 ml: 8 x 6 x 5 cm, Daily Best, Auburn, Australia) containing a single wheat plant (GS14, with the root removed) inserted into 1% agar. A second cup was placed on top and sealed with Parafilm. Sixty replicates were established per treatment: *Rickettsiella*, *Regiella* and wild type. Thirty cups were placed in randomized positions in a temperature-controlled cabinet at 19°C ($\pm$ 1°C) and thirty placed in randomized positions in a temperature-controlled cabinet at 25°C ($\pm$ 1°C). Both cabinets were programmed with a 16:8h L:D photoperiod under plant growth lights. After 7 d, a single aphid was randomly removed and stored in 100% ethanol and frozen for later endosymbiont density measurements. The remaining aphid from each cup was transferred to new cups weekly to assess body color and body shape, fecundity and longevity. The *Rickettsiella* and *Regiella* strains were compared to the wild type in separate experiments, allowing for more replicates to be included.

Body color and body size

To measure the effects of *Regiella* on body color and body size, we removed apterous adults (~ 14 d old) from individual cups containing wheat plants. The aphids that were maintained at both 19°C ($\pm$ 1°C) and 25°C ($\pm$ 1°C) in temperature-controlled cabinets with a 16:8h L:D photoperiod under plant growth lights. We then measured body length and body color by taking photos and analyzing these with ImageJ, as described previously (31). We assessed 25 individuals per strain: *Regiella* and wild type. Different to effects seen in other aphid species (e.g. (15, 31, 41), there was

no obvious effect of *Rickettsiella* on body color after transinfection to *D. noxia*. Nevertheless, to test for subtle differences, we repeated the body color methods above for *Rickettsiella* and wild type aphids, except that we only examined aphids at 19°C ($\pm 1^\circ\text{C}$).

Population dynamics in mixed cages

We assessed the population dynamics of *Rickettsiella* and *Regiella* over multiple generations at two different temperatures. We mixed 30 wild type apterous adults (~ 14 d old) with 30 *Rickettsiella* or 30 *Regiella* apterous adults (~ 14 d old) and transferred these to six wheat plants (~ 6 weeks old), which were placed inside a single BugDorm cage (32.5 x 32.5 x 77 cm, mesh 160 μm aperture, Australian Entomological Supplies, Australia). Each cage was replicated five times and these were placed in randomized positions in a temperature-controlled cabinet at 19°C ($\pm 1^\circ\text{C}$) with a 16:8h L:D photoperiod under plant growth lights. These experiments were repeated under the same conditions, except the cages were placed at 25°C ($\pm 1^\circ\text{C}$). Aphids were transferred to new wheat plants every 3 weeks at 19°C and every 2 weeks at 25°C. During each transfer, 60 randomly selected aphids were placed on the new plants, with the remaining aphids stored in 100% ethanol and frozen for later endosymbiont density measurements. For *Regiella*, the 19°C experiment ran for 24 weeks and the 25°C experiment ran for 30 weeks. The *Rickettsiella* experiments ran for 12 weeks for both temperatures. To measure *Rickettsiella* and *Regiella* frequency over time, we screened 15-20 aphids per replicate cage at multiple time points using qPCR (see “Endosymbiont detection and quantification”).

Population growth and alate production on whole plants

Differences in aphid morphs were observed on barley in the feeding damage experiment, and here we measured aphid population growth and alate production on whole wheat plants. The measurements for *Rickettsiella* and *Regiella* aphids were conducted separately. Different methods were used for the two endosymbionts to reflect specific research aims based on earlier data on aphid morphs and the severity of feeding damage to plants. For *Rickettsiella*, the primary focus was on alate frequency, which was observed to increase at later stages when plants were under stress in the “Feeding damage to barley” experiment. In contrast, for *Regiella*, the emphasis was on early population growth and aphid distribution across the plant due to the potential impact of *Regiella* on reducing damage to plants.

For the *Rickettsiella* experiment, five age-matched apterous adults (14 d old) were placed onto the second true leaf of a single wheat plant (GS14) (see [Figure 3A](#)). Each plant was placed into a BugDorm cage (32.5 x 32.5 x 77 cm, mesh 160 μm aperture) and placed in a randomized position in a temperature-controlled cabinet at 25°C ($\pm 1^\circ\text{C}$) with a 16:8h L:D photoperiod under plant growth lights. Nine replicates were established per treatment: *Rickettsiella* and wild type. Initial survival of aphids was assessed after 24 h and 48 h. At day 14, we removed three replicate plants for both treatments and counted the total number of nymphs, alate adults, and apterous adults. This was repeated again at day 21 and day 25.

For the *Regiella* experiment, five age-matched apterous adults (14 d old) were placed onto each of the top, middle and bottom leaves of a single wheat plant (GS14). A plastic vial was placed over each leaf and cotton wool inserted into the vial opening to prevent aphid movement (see [Figure 4A](#)). Each plant was placed into a BugDorm cage (32.5 x 32.5 x 77 cm, mesh 160 μm aperture) and placed in a randomized position in a temperature-controlled cabinet at 25°C ($\pm 1^\circ\text{C}$) with a 16:8h L:D photoperiod under plant growth lights. Sixteen replicates were established for each treatment: *Regiella* and wild type. Initial survival of aphids was again assessed after 24 h and 48 h. At day 4, we removed four replicate plants for both treatments and counted the total number of nymphs, alate adults, and apterous adults. This was repeated again at day 8, 12 and 16.

Aphid dispersal ability and plant feeding damage in mesocosms

Given the number of alate and apterous adults in our feeding damage experiment on individual plants varied among aphid strains, we undertook a larger-scale experiment to examine the effect of *Rickettsiella* on dispersal ability and plant feeding damage. To do this, three plastic trays (39.5 x

28 x 11cm, Bargain Basement, Australia), each containing eight individual wheat plants (~5-week-old, GS14), were established. These trays were placed inside a large BugDorm cage (93 x 47.5 x 47.5cm, mesh 160 µm aperture), creating a mesocosm environment. Five replicate mesocosms were established and randomly positioned in a temperature-controlled room at 25 (± 1)°C with a 16:8 h L:D photoperiod under plant growth lights. In each mesocosm, *Rickettsiella* and wild type aphids were released at either end of the BugDorm cages, with 5 age-matched apterous adults (14 d old) placed on the second true leaf of each wheat plant ($n = 4$ wheat plants each side) (see [Figure 5A](#)). The four plants with aphids represented the ‘aphid-release’ wheat and the four adjacent plants represented the ‘aphid-spread’ wheat. The middle tray with wheat plants was used to monitor aphid dispersal.

Initial aphid survival was assessed after 24 h and 48 h, and the experiment ran for 5 weeks. Once or twice per week, we recorded the total number of leaves, leaf area, the percentage of leaves with chlorotic streaking, aphid colonization rate, the level of aphid infestation and scored an overall plant damage score (see Table S1) for all ‘aphid-release’ and ‘aphid-spread’ plants (total 16 plants per replicate). All scores were assessed independently and blindly by two observers and then averaged to obtain an overall score for each plant at each time point. If the scores assigned by the two observers differed by more than one unit for a given plant, a third observer independently assessed the plant, and the average score from all three observers was used for the final score. To assess aphid dispersal, all eight wheat plants in the middle ‘dispersal’ tray were removed weekly and replaced with fresh plants at the same plant growth stage. All aphids on these eight plants were subsequently removed, and the number of alate and apterous individuals counted. In addition, 20 alates and 20 apterous aphids at Weeks 2 and 3 within each mesocosm were randomly removed, placed into 100% ethanol and later screened for *Rickettsiella* infection.

Statistical analysis

Life history traits on small plants, aphid population data from large cages, and aphid feeding damage data were analysed using IBM SPSS Statistics 29.0 for Mac following tests for normality. For life history traits on small plants, development time measures were analysed with Kruskal-Wallis Tests. Fecundity was analysed with general linear models (GLMs), with developmental temperature, endosymbiont status (*Rickettsiella*, *Regiella* and wild type) and their interaction included as factors. Endosymbiont density values were compared by independent sample t-tests. We used Cox regression to assess the impact of *Rickettsiella* and *Regiella* on aphid longevity. For body color, body shape, and body size, we analysed each component (hue, saturation, lightness) separately using independent sample t-tests as well as all the components together as a multivariate ANOVA (MANOVA). For the population growth experiment in large cages, GLMs were used to test temporal changes in aphid developmental stage for *Rickettsiella* (Days 21 and 25) and *Regiella* (Days 8–16) aphid strains. Sampling date was log-transformed and normalised prior to analysis. The independent sample t-test was used to compare differences between endosymbiont and wild type treatments at each time point. Early time points were excluded because aphid numbers were similar across all developmental stages at the start of the experiment. For the feeding damage experiments, we used repeated measures in GLMs for the wheat and barley feeding damage experiment and the mesocosm wheat experiment. Aphid dispersal data from the mesocosm experiment was analysed by paired t-tests. Where more than two treatments were compared, we used probabilities from two treatment comparisons (after Bonferroni correction) to determine significance.

All statistical analyses and visualisations for plant responses were conducted in R studio (version 4.5.2) for Mac. Metabolomic data from wheat plant tissues were normalised to the internal standard valine prior to analysis to correct for technical variation. The dataset was normalised by median and log transformed. Principal component analysis (PCA) was performed using all detected components to visualise overall metabolic variation among treatments. Heatmaps were generated using the top 50 components with the highest variance across samples. Differences in all the component abundance between treatments were assessed using linear or generalized linear models, and components with $p < 0.05$ were considered significantly different.

All figures were generated using Prism 10 for Mac and R studio with the ggplot2 and pheatmap packages.

Data availability

All data generated or analysed during this study are included in this submitted article.

Acknowledgements

We thank David de Souza, Jordi Hondrogiannis, Courtney Brown, Ella Yeatman and Kelly Richardson for technical assistance, the Grains Innovative Park for supplying the *D. noxia* colony. This research used NCRIS-enabled Metabolomics Australia infrastructure at the University of Melbourne and funded through BioPlatforms Australia. This work was undertaken as part of the Australian Grains and Horticulture Pest Innovation Program (AGHPIP), supported through funding provided by the Grains Research and Development Corporation (UOM1906-002RTX; UOM2404-006RT), and by Hort Innovation Australia (ST23002) with additional support from the University of Melbourne and Cesar Australia.

Additional files

Supplementary file 1. [Supplementary figures and tables.](#)

Additional information

Funding

Funder	Grant reference number	Author
Grains Research and Development Corporation (GRDC)	UOM1906-002RTX	Paul A Umina
Hort Innovation (HIAL)	ST23002	Ary Hoffman
Grains Research and Development Corporation (GRDC)	UOM2404-006RT	Ary Hoffman

Author ORCID iDs

Xinyue Gu: <https://orcid.org/0000-0003-0591-0356>

Qiong Yang: <https://orcid.org/0000-0003-3336-5703>

References

- Kikuchi Y** (2009) Endosymbiotic bacteria in insects: their diversity and culturability. *Microbes Environ* **24**:195-204 <https://doi.org/10.1264/jsme2.me09140s> | [PubMed](#)
- Moran NA, McCutcheon JP, Nakabachi A** (2008) Genomics and evolution of heritable bacterial symbionts. *Annual Review of Genetics* **42**:165-190 <https://doi.org/10.1146/annurev.genet.41.110306.130119> | [PubMed](#)
- Duron O, et al.** (2008) The diversity of reproductive parasites among arthropods: *Wolbachia* dominates the symbiotic landscape. *Bmc Biology* **6** <https://doi.org/10.1186/1741-7007-6-27> | [PubMed](#)
- Michalkova V, Benoit JB, Weiss BL, Attardo GM, Aksoy S** (2014) Vitamin B6 generated by obligate symbionts is critical for maintaining proline homeostasis and fecundity in tsetse flies. *Applied and Environmental Microbiology* **80**:5844-5853 <https://doi.org/10.1128/aem.01150-14> | [PubMed](#)
- Douglas AE** (1998) Nutritional interactions in insect-microbial symbioses: aphids and their symbiotic bacteria *Buchnera*. *Annu Rev Entomol* **43**:17-37 <https://doi.org/10.1146/annurev.ento.43.1.17> | [PubMed](#)

6. Stillson PT, Martinez K, Adamson J, Tehrani A, Ravenscraft A (2025) Temperature influences outcomes of an environmentally acquired symbiosis. *Isme Journal* **19** <https://doi.org/10.1093/ismejo/wraf056> | PubMed
7. Gu XY, et al. (2022) A w Mel *Wolbachia* variant in *Aedes aegypti* from field-collected *Drosophila melanogaster* with increased phenotypic stability under heat stress. *Environ Microbiol* **24**:2119-2135 <https://doi.org/10.1111/1462-2920.15966> | PubMed
8. Zhang B, Leonard SP, Li YY, Moran NA (2019) Obligate bacterial endosymbionts limit thermal tolerance of insect host species. *Proceedings of the National Academy of Sciences of the United States of America* **116**:24712-24718 <https://doi.org/10.1073/pnas.1915307116> | PubMed
9. Stillson PT, Sim SB, Corpuz RL, Ravenscraft A (2025) Symbiont Gene Expression Predicts Insect Host's Response to High Temperatures. *Molecular Ecology* <https://doi.org/10.1111/mec.70154> | PubMed
10. Pais R, Lohs C, Wu YN, Wang JW, Aksoy S (2008) The obligate mutualist *Wigglesworthia glossinidia* influences reproduction, digestion, and immunity processes of its host, the tsetse fly. *Appl Environ Microb* **74**:5965-5974 <https://doi.org/10.1128/aem.00741-08> | PubMed
11. Rosenwald LC, Sitvarin MI, White JA (2020) Endosymbiotic *Rickettsiella* causes cytoplasmic incompatibility in a spider host. *Proceedings of the Royal Society B-Biological Sciences* **287** <https://doi.org/10.1098/rspb.2020.1107> | PubMed
12. Zabalou S, et al. (2004) *Wolbachia*-induced cytoplasmic incompatibility as a means for insect pest population control. *Proceedings of the National Academy of Sciences of the United States of America* **101**:15042-15045 <https://doi.org/10.1073/pnas.0403853101> | PubMed
13. Hrdina A, Canales MS, Arias-Rojas A, Frahm D, Iatsenko I (2024) The endosymbiont *Spiroplasma poulsonii* increases *Drosophila melanogaster* resistance to pathogens by enhancing iron sequestration and melanization. *Mbio* **15** <https://doi.org/10.1128/mbio.00936-24> | PubMed
14. Massey JH, Newton ILG (2022) Diversity and function of arthropod endosymbiont toxins. *Trends in Microbiology* **30**:185-198 <https://doi.org/10.1016/j.tim.2021.06.008> | PubMed
15. Yu WJ, et al. (2025) A persistent bacterial *Regiella* transinfection in the bird cherry-oat aphid *Rhopalosiphum padi* increasing host fitness and decreasing plant virus transmission. *Pest Management Science* **81**:2791-2799 <https://doi.org/10.1002/ps.8642> | PubMed
16. Shi PQ, et al. (2024) *Rickettsia* transmission from whitefly to plants benefits herbivore insects but is detrimental to fungal and viral pathogens. *Mbio* **15** <https://doi.org/10.1128/mbio.02448-23> | PubMed
17. Yang Q, et al. (2023) A diversity of endosymbionts across Australian aphids and their persistence in aphid cultures. *Environ Microbiol* **25**:1988-2001 <https://doi.org/10.1111/1462-2920.16432> | PubMed
18. Guyomar C, et al. (2018) Multi-scale characterization of symbiont diversity in the pea aphid complex through metagenomic approaches. *Microbiome* **6**:1-21 <https://doi.org/10.1186/s40168-018-0562-9> | PubMed
19. Fukatsu T (1994) Endosymbiosis of aphids with microorganisms: a model case of dynamic endosymbiotic evolution. *Plant Species Biology* **9**:145-154 <https://doi.org/10.1111/j.1442-1984.1994.tb00095.x>
20. Vorburger C, Gehrler L, Rodriguez P (2010) A strain of the bacterial symbiont *Regiella insecticola* protects aphids against parasitoids. *Biology Letters* **6**:109-111 <https://doi.org/10.1098/rsbl.2009.0642> | PubMed
21. Tsuchida T, Koga R, Matsumoto S, Fukatsu T (2011) Interspecific symbiont transfection confers a novel ecological trait to the recipient insect. *Biol Letters* **7**:245-248 <https://doi.org/10.1098/rsbl.2010.0699> | PubMed
22. Kang ZW, et al. (2022) Facultative endosymbiont *Serratia symbiotica* inhibits the apterization of pea aphid to enhance its spread. *Microbiol Spectr* **10**:e04066-04022 <https://doi.org/10.1128/spectrum.04066-22> | PubMed

23. Hansen AK, Moran NA (2014) The impact of microbial symbionts on host plant utilization by herbivorous insects. *Molecular Ecology* **23**:1473-1496 <https://doi.org/10.1111/mec.12421> | PubMed
24. Dedryver CA, Le Ralec A, Fabre F (2010) The conflicting relationships between aphids and men: a review of aphid damage and control strategies. *Cr Biol* **333**:539-553 <https://doi.org/10.1016/j.crv.2010.03.009> | PubMed
25. Valenzuela I, Hoffmann AA (2015) Effects of aphid feeding and associated virus injury on grain crops in Australia. *Austral Entomol* **54**:292-305 <https://doi.org/10.1111/aen.12122>
26. Tatchell GM (1989) An estimate of the potential economic losses to some crops due to aphids in Britain. *Crop Prot* **8**:25-29 [https://doi.org/10.1016/0261-2194\(89\)90095-1](https://doi.org/10.1016/0261-2194(89)90095-1)
27. Edwards OR, Franzmann B, Thackray D, Micic S (2008) Insecticide resistance and implications for future aphid management in Australian grains and pastures: a review. *Aust J Exp Agr* **48**:1523-1530 <https://doi.org/10.1071/ea07426>
28. Reyes ML, et al. (2019) The influence of symbiotic bacteria on reproductive strategies and wing polyphenism in pea aphids responding to stress. *J Anim Ecol* **88**:601-611 <https://doi.org/10.1111/1365-2656.12942> | PubMed
29. Sanches P, De Moraes CM, Mescher MC (2023) Endosymbionts modulate virus effects on aphid-plant interactions. *Isme Journal* **17**:2441-2451 <https://doi.org/10.1038/s41396-023-01549-z> | PubMed
30. Gu XY, et al. (2024) Influence of genetic and environmental factors on the success of endosymbiont transfers in pest aphids. *Environ Microbiol* **26**:e16704 <https://doi.org/10.1111/1462-2920.16704> | PubMed
31. Gu XY, et al. (2023) A rapidly spreading deleterious aphid endosymbiont that uses horizontal as well as vertical transmission. *P Natl Acad Sci USA* **120**:e2217278120 <https://doi.org/10.1073/pnas.2217278120> | PubMed
32. Gu XY, et al. (2025) Transinfections of the endosymbiont *Rickettsiella viridis* in different *Myzus persicae* (Hemiptera: Aphididae) clones show consistent deleterious effects and stable transmission. *Journal of Economic Entomology* **118**:1544-1552 <https://doi.org/10.1093/jee/toaf114> | PubMed
33. Ross PA, et al. (2024) Deleterious effects of the endosymbiont *Rickettsiella viridis* in *Myzus persicae* are environmentally dependent. *J Pest Sci* **98**:375-388 <https://doi.org/10.1007/s10340-024-01786-x>
34. Luna E, et al. (2018) Bacteria associated with Russian wheat aphid (*Diuraphis noxia*) enhance aphid virulence to wheat. *Phyobiomes J* **2**:151-164 <https://doi.org/10.1094/pbiomes-06-18-0027-r>
35. Summers CG, Newton AS, Kirk M, Temple SR (1990) Transmission of beet yellows and beet mosaic viruses by noncolonizing aphid vectors. *J Econ Entomol* **83**:2448-2451 <https://doi.org/10.1093/jee/83.6.2448>
36. Halbert SE, Mink GI, Silbernagel MJ, Mowry TM (1994) Transmission of bean common mosaic virus by cereal aphids (Homoptera: Aphididae). *Plant Dis* **78**:983-985 <https://doi.org/10.1094/PD-78-0983>
37. Ennahli S, et al. (2009) Comparison of categories of resistance in wheat and barley genotypes against biotype 2 of the Russian wheat aphid, *Diuraphis noxia* (Kurdjumov). *Arthropod-Plant Inte* **3**:45-53 <https://doi.org/10.1007/s11829-009-9054-y>
38. Stoetzel MB (1987) Information on and identification of *Diuraphis noxia* (Homoptera: Aphididae) and other aphid species colonizing leaves of wheat and barley in the United States. *J Econ Entomol* **80**:696-704 <https://doi.org/10.1093/jee/80.3.696>
39. Weiland AA, et al. (2008) Biotypic diversity in Colorado Russian wheat aphid (Hemiptera: Aphididae) populations. *J Econ Entomol* **101**:569-574 [https://doi.org/10.1603/0022-0493\(2008\)101\[569:bdicrw\]2.0.co;2](https://doi.org/10.1603/0022-0493(2008)101[569:bdicrw]2.0.co;2) | PubMed
40. Puterka GJ, et al. (2015) Change in biotypic diversity of Russian wheat aphid (Hemiptera: Aphididae) populations in the United States. *J Econ Entomol* **108**:798-804 <https://doi.org/10.1093/jee/tov008> | PubMed
41. Tsuchida T, et al. (2010) Symbiotic bacterium modifies aphid body color. *Science* **330**:1102-1104 <https://doi.org/10.1126/science.1195463> | PubMed

42. Botha AM, Li YC, Lapitan NLV (2005) Cereal host interactions with Russian wheat aphid: a review. *J Plant Interact* **1**:211-222 <https://doi.org/10.1080/17429140601073035>
43. Damsteegt VD, Gildow FE, Hewings AD, Carroll TW (1992) A clone of the Russian wheat aphid (*Diuraphis noxia*) as a vector of barley yellow dwarf, barley stripe mosaic, and brome mosaic viruses. *Plant Dis* **76**:1155-1160 <https://doi.org/10.1094/pd-76-1155>
44. Vierstra RD (1996) Proteolysis in plants: mechanisms and functions. *Plant Mol Biol* **32**:275-302 <https://doi.org/10.1007/bf00039386> | PubMed
45. Malatrasi M, et al. (2006) A branched-chain amino acid aminotransferase gene isolated from *Hordeum vulgare* is differentially regulated by drought stress. *Theor Appl Genet* **113**:965-976 <https://doi.org/10.1007/s00122-006-0339-6> | PubMed
46. Aidoo MK, et al. (2017) A combination of stomata deregulation and a distinctive modulation of amino acid metabolism are associated with enhanced tolerance of wheat varieties to transient drought. *Metabolomics* **13** <https://doi.org/10.1007/s11306-017-1267-y>
47. Mu WM, Yu SH, Zhu LJ, Zhang T, Jiang B (2012) Recent research on 3-phenyllactic acid, a broad-spectrum antimicrobial compound. *Appl Microbiol Biot* **95**:1155-1163 <https://doi.org/10.1007/s00253-012-4269-8> | PubMed
48. Witte CP (2011) Urea metabolism in plants. *Plant Sci* **180**:431-438 <https://doi.org/10.1016/j.plantsci.2010.11.010> | PubMed
49. Coxon KM, et al. (2005) Pantothenate biosynthesis in higher plants. *Biochem Soc T* **33**:743-746 <https://doi.org/10.1042/bst0330743> | PubMed
50. Golan K, et al. (2017) Accumulation of amino acids and phenolic compounds in biochemical plant responses to feeding of two different herbivorous arthropod pests. *Arthropod-Plant Inte* **11**:675-682 <https://doi.org/10.1007/s11829-017-9522-8>
51. Divekar PA, et al. (2022) Plant secondary metabolites as defense tools against herbivores for sustainable crop protection. *Int J Mol Sci* **23**:2690 <https://doi.org/10.3390/ijms23052690> | PubMed
52. Liu XD, Lei HX, Chen FF (2019) Infection pattern and negative effects of a facultative endosymbiont on its insect host are environment-dependent. *Sci Rep-Uk* **9**:4013 <https://doi.org/10.1038/s41598-019-40607-5> | PubMed
53. Zepeda-Paulo F, Villegas C, Lavandero B (2017) Host genotype-endosymbiont associations and their relationship with aphid parasitism at the field level. *Ecol Entomol* **42**:86-95 <https://doi.org/10.1111/een.12361>
54. Bennett AE, Millar NS, Gedrovics E, Karley AJ (2016) Plant and insect microbial symbionts alter the outcome of plant-herbivore-parasitoid interactions: implications for invaded, agricultural and natural systems. *J Ecol* **104**:1734-1744 <https://doi.org/10.1111/1365-2745.12620>
55. Lukasik P, Guo H, Van Asch M, Ferrari J, Godfray HCJ (2013) Protection against a fungal pathogen conferred by the aphid facultative endosymbionts *Rickettsia* and *Spiroplasma* is expressed in multiple host genotypes and species and is not influenced by co-infection with another symbiont. *J Evolution Biol* **26**:2654-2661 <https://doi.org/10.1111/jeb.12260> | PubMed
56. Simon JC, Peccoud J (2018) Rapid evolution of aphid pests in agricultural environments. *Curr Opin Insect Sci* **26**:17-24 <https://doi.org/10.1016/j.cois.2017.12.009> | PubMed
57. Zhang FM, et al. (2015) Bacterial symbionts, *Buchnera*, and starvation on wing dimorphism in English grain aphid, *Sitobion avenae* (F.) (Homoptera: Aphididae). *Front Physiol* **6** <https://doi.org/10.3389/fphys.2015.00155> | PubMed
58. Yang Q, et al. (2026) A *Rickettsiella* transinfection in *Rhopalosiphum padi* reduces fitness and alate production but not plant virus transmission. *Pest Manag Sci* <https://doi.org/10.1002/ps.70508> | PubMed
59. Müller CB, Williams IS, Hardie J (2001) The role of nutrition, crowding and interspecific interactions in the development of winged aphids. *Ecol Entomol* **26**:330-340 <https://doi.org/10.1046/j.1365-2311.2001.00321.x>

60. **Leybourne DJ**, Valentine TA, Bos JIB, Karley AJ (2020) A fitness cost resulting from *Hamiltonella defensa* infection is associated with altered probing and feeding behaviour in *Rhopalosiphum padi*. *J Exp Biol* **223**:jeb207936 <https://doi.org/10.1242/jeb.207936>
61. **Li XA**, et al. (2023) *Sitobion miscanthi* L type symbiont enhances the fitness and feeding behavior of the host grain aphid. *Pest Manag Sci* **79**:1362-1371 <https://doi.org/10.1002/ps.7308> | PubMed
62. **Loudit SMB**, Bauwens J, Francis F (2018) Cowpea aphid-plant interactions: endosymbionts and related salivary protein patterns. *Entomol Exp Appl* **166**:460-473 <https://doi.org/10.1111/eea.12687>
63. **De Jager LE** (2014) Characterization of the mitochondrial genomes of *Diuraphis noxia* biotypes. Stellenbosch University.
64. **Clavé C**, et al. (2022) Physiological costs of facultative endosymbionts in aphids assessed from energy metabolism. *Funct Ecol* **36**:2580-2592 <https://doi.org/10.1111/1365-2435.14157>
65. **Chen YZ**, et al. (2020) An aphid RNA transcript migrates systemically within and is a virulence factor. *P Natl Acad Sci USA* **117**:12763-12771 <https://doi.org/10.1073/pnas.1918410117> | PubMed
66. **du Preez PH**, et al. (2020) DNA methylation and demethylation are regulated by functional DNA methyltransferases and DnTET enzymes in *Diuraphis noxia*. *Front Genet* **11** <https://doi.org/10.3389/fgene.2020.00452> | PubMed
67. **Puterka GJ**, et al. (2014) Characterization of eight Russian wheat aphid (Hemiptera: Aphididae) biotypes using two-category resistant-susceptible plant responses. *J Econ Entomol* **107**:1274-1283 <https://doi.org/10.1603/ec13408> | PubMed
68. **Gu X**, et al. (2024) The endosymbiont *Rickettsiella viridis* increases the virulence of *Diuraphis noxia* to wheat plants and reduces aphid dispersal. *bioRxiv* <https://doi.org/10.1101/2024.09.15.613162>
69. **Ward S**, et al. (2020) Biology, ecology and management of *Diuraphis noxia* (Hemiptera: Aphididae) in Australia. *Austral Entomol* **59**:238-252 <https://doi.org/10.1111/aen.12453>
70. **Dorai APS**, et al. (2024) Novel transinfections of *Rickettsiella* do not affect insecticide tolerance in *Myzus persicae*, *Rhopalosiphum padi*, or *Diuraphis noxia* (Hemiptera: Aphididae). *J Econ Entomol* **117**:1377-1384 <https://doi.org/10.1093/jee/toae136> | PubMed
71. **Niepoth N**, Ellers J, Henry LM (2018) Symbiont interactions with non-native hosts limit the formation of new symbioses. *Bmc Evol Biol* **18** <https://doi.org/10.1186/s12862-018-1143-z> | PubMed
72. **Yazdani M**, et al. (2018) First detection of Russian wheat aphid *Diuraphis noxia* Kurdjumov (Hemiptera: Aphididae) in Australia: a major threat to cereal production. *Austral Entomol* **57**:410-417 <https://doi.org/10.1111/aen.12292>
73. **Chen DQ**, Montllor CB, Purcell AH (2000) Fitness effects of two facultative endosymbiotic bacteria on the pea aphid, *Acyrtosiphon pisum*, and the blue alfalfa aphid, *A. kondoi*. *Entomol Exp Appl* **95**:315-323 <https://doi.org/10.1046/j.1570-7458.2000.00670.x>
74. **Lee SF**, White VL, Weeks AR, Hoffmann AA, Endersby NM (2012) High-throughput PCR assays to monitor *Wolbachia* infection in the dengue mosquito (*Aedes aegypti*) and *Drosophila simulans*. *Appl Environ Microb* **78**:4740-4743 <https://doi.org/10.1128/aem.00069-12> | PubMed
75. **Aldesuquy H**, Baka Z, Mickky B (2014) Kinetin and spermine mediated induction of salt tolerance in wheat plants: Leaf area, photosynthesis and chloroplast ultrastructure of flag leaf at ear emergence. *Egyptian journal of basic and applied sciences* **1**:77-87 <https://doi.org/10.1016/j.ejbas.2014.03.002>

Peer reviews

Reviewer #1 (Public review):

Summary:

In this study, the authors examine what happens when two facultative endosymbionts, *Rickettsiella viridis* and *Regiella insecticola*, are introduced into a novel aphid host, the Russian wheat aphid (*Diuraphis noxia*). They ask whether these introduced symbionts affect

aphid performance, plant damage, alate production, dispersal, plant defense responses, and symbiont dynamics. The main result is that the two symbionts have contrasting effects: *Rickettsiella* tends to increase plant damage and reduce dispersal-related traits, whereas *Regiella* tends to reduce plant damage and aphid population growth, with less evidence for an effect on dispersal.

Strengths:

The manuscript presents successful establishment of stable transinfected populations of an agriculturally important aphid species, which is a substantial technical achievement in itself. I also appreciated that the authors examined the system across several experimental contexts, including different host plants, mixed cages at two temperatures, whole-plant assays, and a mesocosm dispersal experiment, rather than relying on a single laboratory setup. Taken together, these experiments provide a useful and reasonably convincing demonstration that novel symbiont associations can generate contrasting phenotypes in this system.

Weaknesses:

There are some major aspects of this paper that I thought could be strengthened. My main concern is that the manuscript feels broader than it is conceptually focused. A wide range of outcomes is measured, which gives the study breadth, but it also makes the central question harder to identify. As written, the paper reads more strongly as a proof-of-principle demonstration of ecologically relevant phenotypes than as a tightly framed test of a specific biological idea.

A second issue is that the biological basis of the reported phenotypes remains less developed than the phenotypic description itself. The authors make a genuine effort to address mechanism through JA, JA-Ile, SA, and metabolomic profiling, but these analyses only partially explain the main results. The negative result for the canonical defense markers is informative, yet it still leaves a substantial gap between the observed variation in plant damage and the processes responsible for it.

I also think some caution is needed in how the two symbionts are compared. The authors explain why some follow-up experiments were designed differently for *Rickettsiella* and *Regiella*, and that rationale is understandable. Still, because the downstream assays were not fully matched, the paper is strongest when each symbiont is interpreted on its own terms rather than as a strict comparison.

Overall, I would suggest softening the Significance Statement so that it more clearly reflects what is directly shown here, namely that introduced symbionts can alter plant damage and dispersal-related phenotypes under controlled conditions, rather than implying that the study directly tests management utility in agricultural settings.

<https://doi.org/10.7554/eLife.111255.1.sa2>

Reviewer #2 (Public review):

Summary:

The authors generated two novel aphid-symbiont associations and examined the impact of these new symbiotic associations on plant-insect-symbiont interactions. The authors notably provide detailed phenotypic assessments of the insect hosts and host plants. They show that one introduced symbiont, *Rickettsiella*, increases aphid-induced damage to host plants, while the other, *Regiella*, ameliorates aphid damage. The authors suggest that such novel insect-symbiont pairings may be used as tools to mitigate crop damage in the future.

Strengths:

Although a few experiments seem to have limited sample sizes and limited statistical power, these are often complemented with highly replicated smaller-scale experiments. The combination of larger mesocosm and population-level experiments along with assessments of individual insects generally provides a comprehensive depiction of the effects of these symbionts on their hosts. The opposing impacts of *Regiella* and *Rickettsiella* infection on the aphid host plant are of broad interest. It is also surprising that the host plants did not exhibit strong differences in canonical defensive signalling, despite these differences.

Weaknesses:

One thing that I struggled a little with was the rapid spread of *Regiella* in the shared plant experiments. Possibly this could be attributed to an increased reproductive output (due to faster developmental time, and/or an increase in fecundity) or efficient horizontal transmission. However, the other experiments performed indicate a slight negative impact (Figure 4a) or no influence (Figure 4C, 4D, Figure S6) of *Regiella* infection on host fitness. Given these other results, it seems that *Regiella* must spread fairly efficiently between hosts, which comes as a surprise, and there are very few examples of horizontal transmission of *Regiella* like this in the literature. The manuscript would benefit from a clear and direct demonstration of horizontal transmission, rather than it being inferred indirectly. The similar spread observed in the *Rickettsiella* mixed cages is less surprising, because there are several examples where this has been demonstrated.

It is also a little surprising that mesocosm-dispersal experiments were not also conducted using *Regiella*-infected lines. At several points throughout the manuscript, the idea of using symbiont transfections to reduce plant harm is raised. I can understand that these experiments are likely time-, space-, and resource-intensive, but that seems like these would have been relevant experiments, especially in the context of controlling damage to plants.

<https://doi.org/10.7554/eLife.111255.1.sa1>

Reviewer #3 (Public review):

Summary:

The authors were investigating the impact of introducing novel facultative bacterial endosymbionts into the pest aphid, *Diuraphis noxia*, to explore the possibility of using facultative symbionts as a crop protection tool. They successfully established the vertical transmission of both endosymbionts and performed a series of aphid performance and dispersal experiments together with measurement of aphid feeding on host plant health, growth, and metabolism. While most of the experiments revealed no effect of the endosymbionts, some significant treatment effects were found, showing that *Rickettsiella* reduced aphid dispersal, and *Regiella* reduced aphid population growth and feeding damage.

Strengths:

The team worked with two novel facultative symbionts (*Rickettsiella viridis* and *Regiella insecticola*) that they were able to successfully establish in *D. noxia*. The data were collected and analyzed using solid, well-described methodology.

Weaknesses:

While interpretation of the data is reasonable, the few experiments which revealed significant treatment effects rest on relatively small sample sizes.

Measuring symbiont density is difficult. The authors use quantitative PCR to measure the "density" of endosymbionts relative to a host gene. This is a standard approach in the field; however, recent work has shown that endosymbionts like the aphid primary endosymbiont, *Buchnera*, are variably polyploid [1]; further the aphid cells that house the symbionts are also highly polyploid and variable in their ploidy [2]. It is important to understand that what is being measured when using qPCR is DNA copy number and not quantification of the number of symbiont cells. Alternative approaches to measuring symbiont density include flow cytometry [3], and SymbiQuant [4], a machine vision tool that can quantitatively characterize symbiont populations from DAPI-stained confocal images. These alternate approaches also have their limitations. Currently, there is no perfect approach to measuring symbiont density, which remains an important measure in experiments such as these. Put simply, it is important for a reader to be aware of the limitations of each approach and interpret data accordingly.

[1] Komaki, K., and H. Ishikawa. 2000. Genomic copy number of intracellular bacterial symbionts of aphids varies in response to developmental stage and morph of their host. *Insect Biochemistry and Molecular Biology* 30:253-258.

[2] Nozaki, T., and S. Shigenobu. 2022. Ploidy dynamics in aphid host cells harboring bacterial symbionts. *Scientific Reports* 12:9111.

[3] Simonet, P., G. Duport, K. Gaget, M. Weiss-Gayet, S. Colella, G. Febvay, H. Charles, J. Viñuelas, A. Heddi, and F. Calevro. 2016. Direct flow cytometry measurements reveal a fine-tuning of symbiotic cell dynamics according to the host developmental needs in aphid symbiosis. *Scientific Reports* 6:19967.

[4] James, E. B., X. Pan, O. Schwartz, and A. C. C. Wilson. 2022. SymbiQuant: A machine learning object detection tool for polyploid independent estimates of endosymbiont population size. *Frontiers in Microbiology* 13:816608.

Impact and Significance:

Food security and production, and pest control are major challenges facing the human population. This work contributes knowledge that will benefit the development of alternate pest control strategies in agriculture.

<https://doi.org/10.7554/eLife.111255.1.sa0>