

Water deficit and aphid resilience on wheat: examining *Sitobion avenae* F. and their bacterial symbionts interplay under controlled laboratory conditions

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Abstract

BACKGROUND: Climate change has far-reaching effects on food security and agriculture, affecting crop yields and food distribution. Agriculture relies heavily on water for irrigation and production, making it vulnerable to water scarcity. Additionally, climate change can affect crop pest insects, leading to increased global crop losses, particularly in cereals, an important component of the human diet. Aphids are major crop pests and have a symbiotic relationship with bacterial endosymbionts that can contribute to their success as pests under a climate change scenario. To test the effect of drought on aphids, we examined varying levels of water deficit and endosymbiont composition on the grain aphid (*Sitobion avenae*) performance on wheat under controlled laboratory conditions. We measured the intrinsic rate of population increase (r_m), the body weight of adult aphids, and the pre-reproductive period for different genotypes of the grain aphid (including Chilean superclones) under different irrigation regimes. We also analyzed the relative abundance of their endosymbionts under the different water treatments.

RESULTS: Our findings revealed that water deficit affects each aphid genotype differently, impacting various traits. For instance, the body weight of adult aphids was notably affected by different water treatments, with aphids grown under intermediate water deficit (IW) being significantly bigger. The relative abundance of endosymbionts also varied among genotypes and water treatments—specifically *Regiella insecticola* had a noticeably higher abundance under IW ($P < 0.05$).

CONCLUSION: This study provides valuable insights into the impact of water deficit on aphid performance and the role of endosymbionts in mitigating the effects of water deficit.

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Supporting information may be found in the online version of this article.

Keywords: water deficit; *Sitobion avenae*; endosymbionts; aphid superclones; wheat

1 INTRODUCTION

The impact of climate change on food security and sustainable agriculture is becoming increasingly concerning.¹ Global warming, drought, climate variability, and extreme weather conditions are significantly affecting crop yields, food distribution, and overall food security.² Agriculture, which heavily relies on water for irrigation and production processes, is particularly vulnerable to water scarcity, which can have severe implications for food security.³ In fact, in 2017, agriculture accounted for 83% of the total damage and loss resulting from drought in 53 developing countries.⁴

Drought seriously threatens crop yields and is considered the most devastating stress in agriculture. It produces water deficits and salinity problems that hinder plant photosynthesis, respiration, and stomatal movement, thereby impacting their growth

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and physiological metabolism, as well as their phenology and weakening their chemical defenses against insect pests.^{5–9}

Directly or indirectly, climate change significantly impacts insect pests, affecting their biology, distribution, outbreak, and aggressivity in unpredictable and extensive ways.¹⁰ Directly, climate change—through phenomena such as drought, increased temperatures and atmospheric CO₂, unstable climates, and altered frequency/intensity of extreme weather events—creates conditions that favor the arrival and spread of invasive species.^{11,12} Indirectly, climate change also disrupts the synchronization between interacting species in agroecosystems, as plants, insect herbivores, and their natural enemies respond differently to climate change,¹³ posing a significant threat to food security when suitable control strategies are lacking.¹⁴

Aphids (Hemiptera: Aphididae) are insect herbivores that constitute major crop pests worldwide due to their (i) clonal reproduction and telescoping of generations, which build up large population sizes quickly reaching economic thresholds. (ii) The ability to transmit plant viruses and (iii) multiple insecticide resistance mechanisms.¹⁵ Remarkably, functional symbioses with a range of bacterial endosymbionts have gained evidence to explain the success of aphids as pests. Endosymbiotic relationships are widespread in animals, particularly arthropods.¹⁶ Aphids harbor an obligated (or primary) bacterial endosymbiont, *Buchnera aphidicola*, which is critical for aphid survival as it provides essential amino acids and vitamins the aphids cannot obtain from the plant sap.¹⁷ Additionally, aphids can harbor facultative (or secondary) non-essential bacterial endosymbionts, which have been shown to affect their life history traits such as reproduction and body mass,^{18,19} and provide them with adaptative advantages such as thermal tolerance, protection from natural enemies, and expanded host-plant range.^{20–23}

The grain aphid *Sitobion avenae* Fabricius is one of the most invasive cereal pests worldwide,²⁴ and one of the most frequent pests of wheat crops in Chile.²⁵ *S. avenae* presumably originated from Eurasia and was introduced to Chile in the late 1960s.²⁶ It significantly reduced wheat yields (>40%) and led to regular insecticide applications in Chile.²⁷ In the late 1970s, parasitoid wasps were introduced to control the grain aphid, resulting in reduced insecticide use,²⁸ which continues to be practiced in Chile.

Despite the wide geographical distribution of *S. avenae* in Chile, its population is predominantly comprised of a few genotypes that account for 80% of the total genotypic diversity.²⁹ These genotypes, referred to as superclones, are highly frequent and widely distributed.³⁰ The rapid spread and invasiveness of *S. avenae* would result from combining obligate parthenogenetic reproduction all year round, enabling them to rapidly spread through a territory, along with its phenotypic plasticity due to vertically transmitted bacterial endosymbionts.^{15,25} *S. avenae* is primarily infected by the facultative endosymbionts *Regiella insecticola* and *Hamiltonella defensa*,^{31–34} which have been associated with improved adaptative responses under stress, such as defense against parasitoids and entomopathogenic fungi.^{19,35} Interestingly, *R. insecticola* has been shown to be present in high frequencies in central Chile, exceeding 90%.³¹

Recent studies suggest that facultative endosymbionts in aphids can help in mitigating the impacts of climate change,^{36,37} particularly concerning increased temperature.^{38–40} However, the potential role of facultative endosymbionts in a water deficit scenario has not been explored. Furthermore, it remains unclear whether the presence of facultative endosymbionts in

superclones could lead to a more effective response compared with less common genotypes under such conditions.

Hence, this study investigated how aphids raised on wheat seedlings growing in water-deficient conditions under controlled laboratory conditions respond. First, we investigated changes in aphid life-history traits, as they are closely tied to the fitness of aphids grown on wheat plants with different irrigation regimes. We used two superclone genotypes of the grain aphid infected with *R. insecticola*, the most frequent facultative endosymbiont in Chile, and a non-superclone, infected with *H. defensa*. We wanted to explore whether the presence or absence of facultative bacterial endosymbionts alters the aphid responses to water-deficient conditions on wheat. Second, we also aimed to investigate the impact of different water regimes on the abundance of obligate and facultative symbionts to better understand the internal interaction between aphids and their endosymbionts in response to water deficit. Finally, we discussed the importance of aphid responses and the presence of endosymbionts in aphid outbreaks caused by drought and their impact on trophic interactions with aphid natural enemies.

2 MATERIALS AND METHODS

2.1 Aphid lineages, genotypes, and endosymbiont selection

During the 2016 wheat growing season (spring) in Chile, live aphids of *S. avenae* were collected from wheat crops. Subsequently, they were transported to the laboratory and individually placed inside separate tubes with a wheat plant. The top of each tube was covered with a Terylene mesh. These aphid-plant systems were maintained under controlled laboratory conditions at a temperature of 21 °C and a photoperiod of 16 h of light and 8 h of darkness, which allowed for the successful parthenogenetic reproduction of the aphids (i.e., only females were produced).

To determine the genotypes of the aphids, we employed a multiplex PCR kit (Correa *et al.* in prep.) that amplified seven microsatellite loci (Sm12, S3.43, S19, S5.L, Sm17, Sm10, and S3.R).^{41,42} This kit enabled us to amplify all seven loci in a single PCR reaction. The amplified fragments were then sized using an automated capillary sequencer (ABI 3700 sequencer, Applied Biosystems), and the results were analyzed with GeneMarker v1.75 (SoftGenetics LLC). Furthermore, we utilized specific markers to determine the presence of the primary endosymbiont, *Buchnera aphidicola*, as well as the seven most commonly found facultative endosymbionts in aphids, including *Hamiltonella defensa*, *Regiella insecticola*, *Serratia symbiotica*, *Spiroplasma* sp., *Rickettsia* sp., *Rickettsiella viridis*, and *Fukatsuia symbiotica*.^{31,34,43}

The frequency of *S. avenae* genotypes and the presence of facultative endosymbionts in Chile have been previously determined.⁴⁴ Based on that report, we chose the two most frequent Chilean superclones, Sa2 and Sa3, which were found to only host *R. insecticola* as a facultative endosymbiont. Due to the absence of live aphid genotypes from Chile carrying *H. defensa* in the laboratory, we opted for a third genotype (SaF16) from France (non-superclone) with the facultative endosymbiont *H. defensa*, which was already in the laboratory.⁴⁴

To properly evaluate the effects of a particular endosymbiont on aphid performance, it is necessary to compare infected and uninfected host insects with highly similar genetic backgrounds. Most *S. avenae* individuals in Chile carry *R. insecticola*,^{31,34} so uninfected genotypes could not be obtained from naturally

endosymbiont-free individuals. A common method to obtain uninfected aphid individuals involves using antibiotics to eliminate infections with facultative symbionts in individuals and generate cured and infected individuals of the same genotype.^{45,46} Hence, a combination of antibiotics (ampicillin, cefotaxime, and gentamicin) was administered via a 30% sucrose artificial diet to cure the aphid genotypes of their endosymbionts.⁴⁷ Following a successful disinfection spanning over 10 generations, infected (E+) and cured (E−) aphid lines were obtained and used for further experiments in this study.

2.2 Plants and water deficit experiments

Wheat seedlings (*Triticum aestivum* L.) cultivar Millán (INIA-Chile) were used to study the impact of water deficit on aphids. This particular cultivar is widely grown in Chile and is best suited for irrigated soils,^{48,49} making it susceptible to the effects of water deficit. The experiments involved sowing three seeds in 4 cm-diameter pots filled with 7 g of a substratum mixture of turfy soil, vermiculite, and perlite (4:3:1).⁵⁰ Turfy is a lightweight organic growing medium that can absorb 10 to 20 times its weight in water.⁵¹ Perlite and vermiculite are inorganic growing media providing optimal aeration and increased drainage for root development.⁵¹ The pots were saturated with water after sowing and allowed to drain for 24 h. The weight of each pot after draining served as the baseline for treatments (100%).⁵² Three distinct irrigation conditions were established for the experiment: the control condition, known as 'well-watering' (WW), which involved maintaining the pot's weights between 80% and 100% of the baseline; an 'intermediate watering' (IW) condition, set between 50% and 60% of weight, and a 'low watering' (LW) condition, established between 30% and 40% of weight.⁵⁰ Plants were weighed daily and watered three times a week with a carefully measured volume of water to maintain the desired irrigation ranges according to the weight. This watering schedule was implemented 5 days after the sowing and continued for 16 days (meaning 21 days after the sowing).

After 10 days of water treatment (15 days post-sowing), one 24 h-old individual aphid nymph (immature stage) of each genotype (Sa2, Sa3, and SaF16) and endosymbiont composition, infected (E+) or cured (E−) was introduced on plant seedlings, enclosed within a cellophane bag with small holes to allow gas exchange, where the respective water treatment was applied (WW, IW or LW). Each plant-aphid system was placed in climatic rooms at 21 °C, with a photoperiod of 16 h of light and 8 h of darkness. To prevent any negative effects on aphids from plant deterioration, aphid individuals were transferred to fresh plants (15 days post-sowing) with the same irrigation regimen every 21 days until the completion of the experiments. Additionally, to evaluate the impact of the different water treatments on the experimental wheat plants, we assessed the stomatal conductance of 15-day-old plants using the portable Leaf Prometer device (SC-1; Decagon Devices, Inc., Pullman, WA, USA) (Supplementary Data S1).

2.3 Life history traits

Life history traits have proven to be reliable indicators of aphid performance. Particularly, the intrinsic rate of population increase (r_m),⁵³ has been widely used as a reliable measure of aphid performance because it includes both developmental and reproductive rates.⁵⁴ Therefore, to investigate the effects of water deficit and endosymbionts on grain aphids, we conducted a study measuring the r_m , adult body weight, and pre-reproductive time, traits that

have been shown to vary significantly according to the presence of facultative endosymbionts,^{55–57} of aphids reared on wheat seedlings treated with different irrigation regimes. This was done by taking a single 24-h-old nymph set on a wheat plant for every watering treatment and confined in a plant system. Each aphid nymph was observed daily until the pre-oviposition time (i.e., time to first reproduction) (Td) when aphids are considered to have reached adulthood. Then, newborn nymphs were counted (Md) for a time equal to Td, and the number of offspring was used to calculate the r_m of each caged aphid, $r_m = 0.738 (\ln Md)/Td$, where 0.738, is a correcting constant, calculated from the slope of Md over Td.⁵³ As aphids reproduce through clonal reproduction with telescoping generations, they may transmit stressful signals to their offspring, which can alter the life history traits of daughter nymphs from one generation to the next.⁵⁸ To investigate this, we weighted each aphid at adulthood across two generations (G1 and G2) under the varying watering treatments using a Mettler Toledo MX5 Micro Balance. Ten biological replicates were conducted for every life-history trait studied in each water treatment, aphid genotype, and endosymbiont presence.

2.4 DNA extraction and quantification of the relative abundance of bacterial endosymbionts

As aphids from the first generation (G1) were utilized to measure their life-history traits, the molecular studies focused on the second generation (G2). Hence, nymphs of 24 h old from G2 were allowed to grow until their first reproduction on wheat seedlings with the same watering regime that those experienced by their mothers (G1). Then, three individual aphids from each genotype/facultative endosymbiont composition/watering regime combination were randomly selected and kept separately in an Eppendorf tube with 95% alcohol until DNA extraction, which was done by the 'salting out' method.⁴¹

We developed specific primers for the molecular identification of *B. aphidicola* and *R. insecticola*. We measured the relative abundance of facultative bacteria *H. defensa* and *R. insecticola*, as well as the obligate endosymbiont *B. aphidicola*, relative to the aphid host, by using a gene of single copy (elongation factor 1 α (EF-1 α)) (Table 1). To target *B. aphidicola*, we utilized the GroEL sequence from the genome of a Chilean superclone of *S. avenae*⁵⁹, and for *R. insecticola*, we identified an appropriate GyrB sequence from the literature.⁶⁰ The primer sequences for *H. defensa* were taken from a research paper (Table 1).⁶¹ These specific primers were employed for quantitative PCR (qPCR) to screen the relative abundance of bacterial endosymbionts. A series of *S. avenae* DNA dilutions were conducted at a 1:4 ratio to assess the efficiency of primers used for endosymbiont relative abundance calculations. A total of six dilutions were prepared, starting with an initial concentration of 100 ng/ μ L and ending with a final concentration of 0.094 ng/ μ L. The primer efficiency percentage was calculated using the following formula: Efficiency = $(10^{(-1/\text{slope})} - 1) \times 100$. Three biological samples, each consisting of a single aphid DNA, and three technical replicates were utilized in the analysis.

The amplification was carried out in a Stratagene Mx 3000 P (Agilent Technologies) real-time thermocycler. To provide a brief overview, we utilized one μ L of DNA (25 ng/ μ L), 10 μ L of ROX SYBR 2X Master Mix dTTP Blue (Takyon™), 0.4 μ L of forward and reverse primers (10 pmol), and 8.2 μ L of ultrapure water, resulting in a total volume of 20 μ L. The amplification conditions were as follows: one cycle of 95 °C for 10 min (polymerase activation);

Table 1. Primer sequences used for qPCR

Target	Gen	Primer	Sequence (5'-3')	Amplicon size (bp)	Source
<i>Buchnera aphidicola</i>	<i>GroEL</i>	Forward	TCGGAACGAAGCCCGAAT	204	This study
		Reverse	CTTTTACCATTTGCGCACCC		
<i>Regiella insecticola</i>	<i>GyrB</i>	Forward	GAAGTGAAAACCGCCGTTGA	167	This study
		Reverse	TCTAAAGCGCCTTTACGCCT		
<i>Hamiltonella defensa</i>	<i>GyrB</i>	Forward	CGCAAGGCAGTCATTATATTTTTG	201	Clarke 2013 ¹⁰⁴
		Reverse	GACAGATTTTTTGATATTCGCTACTTTG		
<i>Elongation factor 1α</i>	<i>EF-1α</i>	Forward	CTGATTGTGCCGTGCTTATTG	160	Oliver et al. 2003 ⁶¹
		Reverse	TATGGTGGTTCAGTAGAGTCC		

40 cycles of 94 °C for 30 s and 60 °C for 30 s; 72 °C for 1 min; and finally, a dissociation curve consisting of 1 min at 95 °C, 30 s at 60 °C, and 30 s at 95 °C, followed by 1 min at 72 °C. Ultra-pure water was used as a negative contamination control (No Template Control, NTC). The relative abundance of endosymbiotic bacteria was normalized to the reference gene (*EF-1 α*) and calculated using the mean of CT and $2^{-\Delta\Delta Ct}$.⁶²

2.5 Data analysis

The effects of different watering treatments on the measured variables, r_m , body weight of adult aphids, the pre-reproductive period, and endosymbiont abundance, were assessed using separate models. To evaluate the effects of different watering treatments, aphid genotype, and endosymbiont presence on r_m and body weight of adult aphids, we utilized mixed linear models (LMM) through the R Studio Cloud platform.⁶³ The response variables were r_m and body weight of adult aphids (G1 and G2). When the data did not adhere to a normal distribution, we applied a log transformation for the body weight of adult aphids and a Box-Cox transformation for r_m .⁶⁴ To create more understandable data visualizations, we plotted graphs using non-transformed data (i.e., raw data). The pre-reproductive time (i.e., time to the first reproduction) was analyzed using Cox's proportional hazard model.⁶⁵ The proportional hazards assumption was checked using the *cox.zph* function from the survival package.⁶⁶ The fixed factors included the watering treatment (WW, IW, and LW), endosymbiont presence (E+ and E-), and aphid genotype (Sa2, Sa3, and SaF16), while the random factor was the temporal block (4 temporal blocks; 3, 2, 2, and 3 replicates per block, respectively). The significance of fixed factors and their interaction was tested with an analysis of deviance (Type II tests).⁶⁷

Variance comparisons of the abundance of the symbiont bacteria *R. insecticola*, *H. defensa*, and *B. aphidicola* were performed using ANOVA in R Studio, with the response variable being the abundance of each bacterium ($2^{-\Delta\Delta Ct}$).⁶² We conducted a deviance analysis to determine the significant effects of the fixed factors studied. All *post hoc* comparisons were made using the *emmeans* package in R Studio using the Tukey method.⁶⁸

3 RESULTS

3.1 Performance of *Sitobion avenae* aphids reared on wheat seedlings subjected to different watering regimes

3.1.1 Intrinsic rate of population increase (r_m)

After conducting experiments on different aphid genotypes under the different watering treatments studied, we observed that both the aphid genotype and endosymbiont composition play a significant role, along with their interaction (Table 2). The

genotype SaF16 displayed a smaller r_m (0.215 ± 0.005) compared with Sa2 (0.243 ± 0.003) and Sa3 (0.238 ± 0.003), while cured aphids (E-) showed a significantly higher r_m (0.238 ± 0.002) than the infected (E+) (0.225 ± 0.004). Although the water treatment factor alone was statistically non-significant (WW = 0.226 ± 0.004 ; IW = 0.237 ± 0.003 ; LW = 0.234 ± 0.004), its interaction with the aphid's endosymbiont was significant (Table 2). This indicated that the r_m in E- aphids (0.248 ± 0.003) was significantly higher than in E+ individuals (0.225 ± 0.005) under IW. The interaction of aphid genotype and endosymbiont composition was only observed in the genotype SaF16, where E- individuals showed a higher r_m than E+ (Fig. 1(C)). No such interaction was found for Sa2 (Fig. 1(A)) and Sa3 (Fig. 1(B)). The complete data for the total number of nymphs and the pre-reproductive period is available in Supplementary S2.

3.1.2 Body weight of adult aphids

Based on the data, the body weight of adult aphids at G1 was not significantly affected by either the aphid's genotype (Sa2 = 0.685 ± 0.034 ; Sa3 = 0.598 ± 0.029 ; and SaF16 = 0.641 ± 0.032) or endosymbiont composition alone (E+ = 0.630 ± 0.027 , E- = 0.652 ± 0.025); however, their interaction was significant (Table 2). The significance was not found in genotype Sa2 (E+ = 0.732 ± 0.053 ; E- = 0.637 ± 0.042); and Sa3 = (E+ = 0.599 ± 0.047 ; E- = 0.596 ± 0.035) only in genotype SaF16, which exhibited a higher weight in E- individuals (0.723 ± 0.049) than E+ individuals (0.559 ± 0.036). Furthermore, the water treatment had a notable impact on the aphid's weight, although it did not interact with other factors (Table 2). The difference between water treatments was due to a significantly larger weight of aphids in IW (0.685 ± 0.031) than in WW (0.586 ± 0.028), although neither differed from LW (0.653 ± 0.036). The endosymbiont composition and water treatment did not significantly affect the weights of genotypes Sa2 (Fig. 2(A)), Sa3 (Fig. 2(B)), and SaF16 (Fig. 2(C), Table 2).

In the second generation (G2), a similar outcome to that of G1 was observed. The aphid's weight was not influenced by its genotype (Table 2) (Sa2 = 0.730 ± 0.039 ; Sa3 = 0.665 ± 0.036 and SaF16 = 0.646 ± 0.038). However, the presence of the facultative endosymbiont had a significant impact on the aphid's weight. The E- individuals exhibited a much larger weight (0.721 ± 0.032) than E+ (0.639 ± 0.029). Additionally, the water treatment affected the aphid's weight (Table 2), with IW (0.751 ± 0.042) showing a higher weight compared with WW (0.586 ± 0.028), but both were not significantly different from LW (0.691 ± 0.039). The interaction of genotype and endosymbiont composition was also significant (Table 2); adult aphids from genotype

Table 2. Deviance table for the life-history traits measured in different genotypes of *S. avenae* and endosymbiotic composition subjected to different watering regimes

Factor	df	r_m		Aphid weight G1		Aphid weight G2		Pre-reproductive period	
		χ^2	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	χ^2	<i>P</i>
Aphid Genotype	2	24.814	<0.001	1.954	0.146	4.377	0.112	25.847	<0.001
Endosymbiont	1	10.260	0.001	0.745	0.389	6.177	0.013	3.294	0.069
Water treatment	2	5.032	0.081	3.164	0.045	12.988	0.001	1.058	0.589
Genotype: Endosymbiont	2	35.627	<0.001	3.967	0.021	21.718	0.019	6.897	0.032
Genotype: Water treatment	4	1.037	0.904	0.424	0.791	4.946	0.292	2.005	0.735
Endosymbiont: Water treatment	2	6.852	0.032	1.681	0.190	0.3816	0.826	3.812	0.148
Genotype: Endosymbiont: Water treatment	4	2.800	0.592	0.513	0.726	2.366	0.668	1.196	0.878

df, degrees of freedom; χ^2 , Chi-square value; *F*, F-value of ANOVA; *P*, *P*-value of the test. Bold values indicate significant *P*-values (*P* < 0.05).

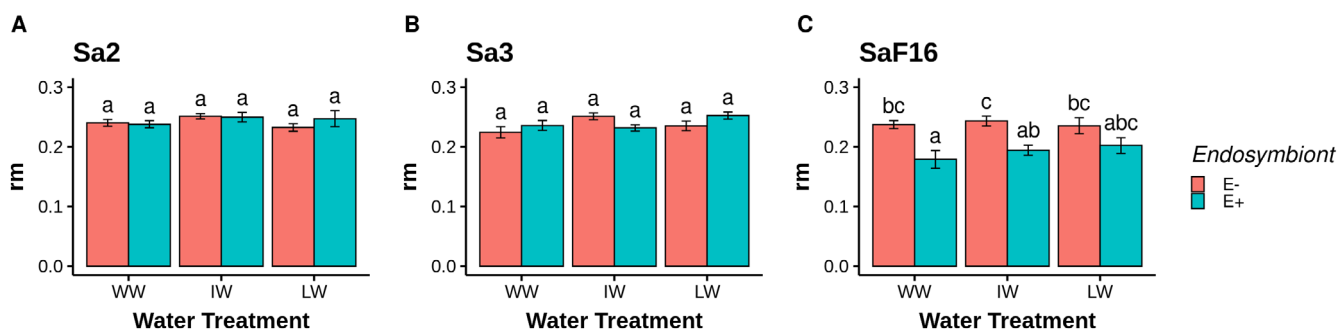


Figure 1. The intrinsic rate of population increase (r_m) in different genotypes, endosymbiont compositions, and water treatments. Different letters over the bars indicate significant differences (*P* < 0.05).

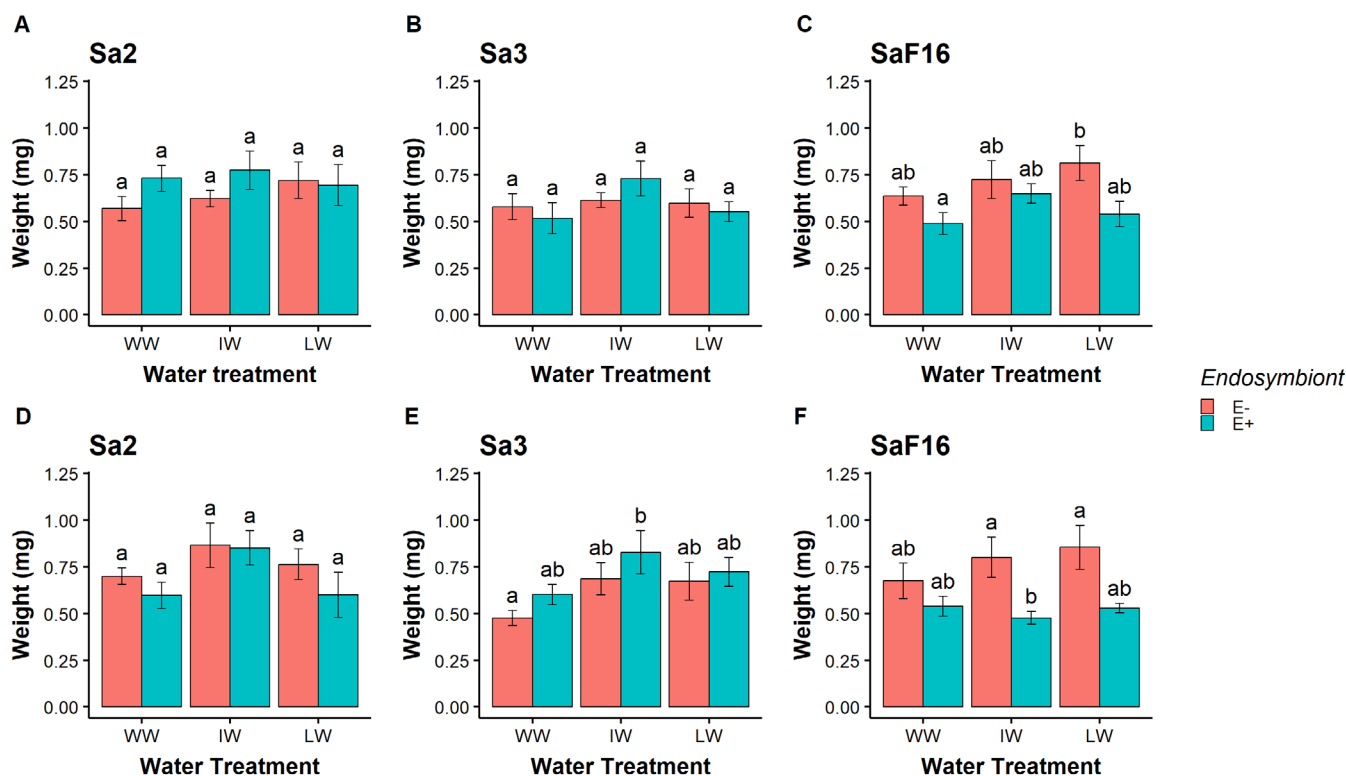


Figure 2. Aphid weight from generations G1 and G2 in different genotypes, endosymbiont composition, and water treatments. G1 (A, B, and C), G2 (D, E, and F). WW, well-watered (control); IW, intermediate watering; LW, low watering. Different letters over the bars indicate significant differences (*P* < 0.05).

SaF16 showed a lower weight when they were infected with *H. defensa* ((E+) = 0.515 ± 0.022) than when they were cured of endosymbiont ((E-) = 0.776 ± 0.061), but that pattern was not significant for Sa2 ((E+) = 0.683 ± 0.059 ; ((E-) = 0.611 ± 0.049)) and Sa3 ((E+) = 0.718 ± 0.052 ; ((E-) = 0.612 ± 0.049)). The aphid genotype and water treatment interaction was also significant, influenced mainly by the SaF16 genotype; the E- individuals had a larger weight than E+ individuals, regardless of the water treatment (Fig. 2(F)), a pattern not observed for genotypes Sa2 (Fig. 2(D)) and Sa3 (Fig. 2(E)).

Finally, when comparing aphid weight between G1 and G2, there was no effect of the generation factor ($F = 2.023$, $df = 1$, $P = 0.156$), its interaction with the water treatment ($F = 0.371$, $df = 2$, $P = 0.691$), aphid genotype ($F = 0.427$, $df = 2$, $P = 0.652$), or endosymbiont composition ($F = 1.196$, $df = 1$, $P = 0.275$) (see the complete ANOVA table in Supplementary S3).

3.1.3 Pre-reproductive period (Td)

Table 3 presents the range of days of the pre-reproductive period (Td), which varied between 8 and 15 days. According to the findings, the aphid's genotype significantly impacted the pre-reproductive period, as did the interaction between the genotype and the endosymbiont composition. Specifically, individuals from the SaF16 genotype took longer to reproduce than those from other genotypes, with the most significant delay observed in E+ individuals from SaF16 (as illustrated in Table 3). However, the water treatment and its interactions with other factors did not affect the pre-reproductive period significantly (Table 2).

3.1.4 Endosymbiont relative abundance

The primer efficiencies for *R. insecticola*, *H. defensa*, *B. aphidicola*, and *S. avenae* elongation factor were 98%, 96%, 100%, and 97%, respectively (CT values in Supplementary S4). Determining the relative abundance of the obligate and facultative endosymbionts in each aphid genotype began with examining their relative abundance under normal irrigation conditions (WW). This assessment aimed to identify any inherent differences in endosymbiont prevalence among the studied genotypes under well-watered regimes. Our findings indicated that there was a significant effect of genotype on the abundance of *B. aphidicola* ($F = 23.825$, $df = 2$, $P < 0.001$), as well as facultative endosymbiont presence ($F = 4.511$, $df = 1$, $P = 0.039$). Superclone Sa2 had a higher relative abundance of *B. aphidicola* than Sa3, regardless of whether it was infected or cured of *R. insecticola* (Fig. 3(A)). Interestingly, only the genotype SaF16 had a higher relative abundance of

B. aphidicola when infected with its facultative endosymbiont (E+), *H. defensa*, than when cured (E-) (Fig. 3(A)). Significant differences were found when comparing the relative abundance of *R. insecticola* between genotypes Sa2 and Sa3 ($F = 8.77$, $df = 1$, $P = 0.018$). Sa2 displayed a significantly higher proportion of *R. insecticola* than Sa3. Conversely, SaF16 exhibited a significantly higher proportion of *H. defensa* than genotypes infected with *R. insecticola* (Fig. 3(B)).

After analyzing the effects of different irrigation regimes and endosymbiont composition on the relative abundance of endosymbionts for each genotype, it was found that in genotype Sa2, the water treatment ($F = 3.038$, $df = 2$, $P = 0.057$) and endosymbiont composition ($F = 1.563$, $df = 1$, $P = 0.217$) did not have an individual effect on the abundance of *B. aphidicola*. However, a significant interaction between both factors ($F = 5.589$, $df = 2$, $P = 0.006$) was observed, indicating a significantly higher abundance of *B. aphidicola* in treatment IW when aphids were infected with *R. insecticola* (Fig. 4(A)).

The relative abundance of *B. aphidicola* was significantly influenced by the water treatment in the Sa3 genotype ($F = 4.528$, $df = 2$, $P = 0.0158$). The IW treatment showed a higher relative abundance, particularly in the presence of *R. insecticola* infection (Fig. 4(C)). However, neither the endosymbiont ($F = 0.155$, $df = 1$, $P = 0.695$) nor the interaction of both factors ($F = 1.689$, $df = 2$, $P = 0.195$) had a significant effect.

On the other hand, the abundance of *B. aphidicola* in the SaF16 genotype was not affected by either the water treatment ($F = 0.265$, $df = 2$, $P = 0.768$) or the presence of endosymbionts ($F = 3.735$, $df = 1$, $P = 0.059$) alone. Nevertheless, the interaction between both factors had a significant effect ($F = 8.038$, $df = 2$, $P = 0.001$), resulting in a higher abundance of *B. aphidicola* for individuals infected with the secondary endosymbiont *H. defensa* under the IW regime (Fig. 4(E)).

When the abundance of facultative endosymbionts in infected aphids was measured for each water treatment, the data showed that the water treatment had a significant effect ($F = 16.25$, $df = 2$, $P < 0.001$) in genotype Sa2, with a significantly higher abundance of *R. insecticola* in the IW water treatment compared with WW and LW (Fig. 4(B)). A similar result was observed for genotype Sa3 ($F = 5.158$, $df = 2$, $P = 0.013$), with a higher abundance of *R. insecticola* in the IW water treatment than WW, but it was not different from LW (Fig. 4(D)). Finally, the water treatment did not significantly affect the abundance of *H. defensa* in genotype SaF16 ($F = 2.966$, $df = 2$, $P = 0.070$) (Fig. 4(F)).

Table 3. Pre-reproductive period for genotype and endosymbiont presence

Genotype	Endosymbiont	Pre-reproductive period	
		<i>n</i>	Mean \pm SE
Sa2	E-	30	9.9 \pm 0.117 a
Sa2	E+	29	9.6 \pm 0.159 a
Sa3	E-	30	9.6 \pm 0.146 a
Sa3	E+	30	9.8 \pm 0.154 a
SaF16	E-	30	10.0 \pm 0.195 a
SaF16	E+	30	11.1 \pm 0.246 b

n, number of individuals analyzed. Different letters next to mean values indicate significant differences ($P < 0.05$).

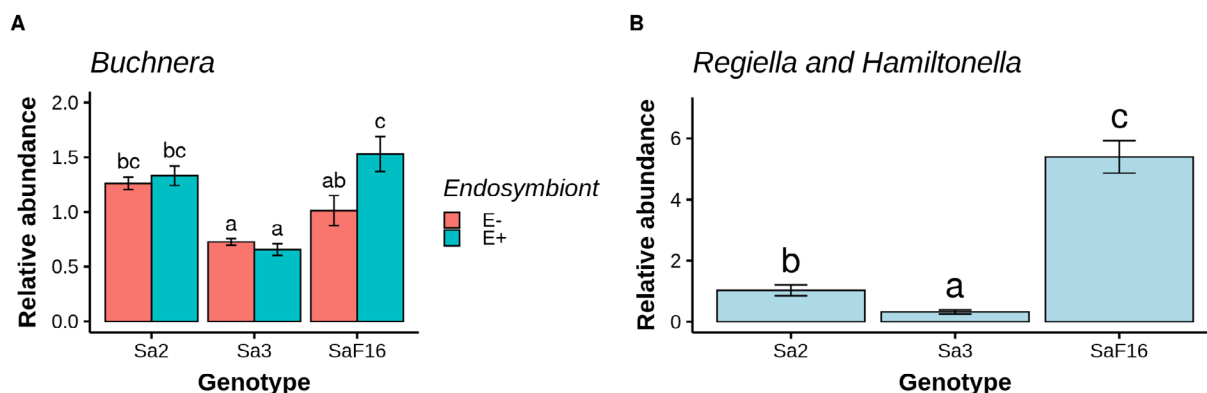


Figure 3. Relative abundance of endosymbionts in different genotypes under well-watered conditions (WW). *Buchnera aphidicola* (A), *Regiella insecticola* (Sa2 and Sa3), and *Hamiltonella defensa* (SaF16) (B). Different letters over the bars indicate significant differences ($P < 0.05$).

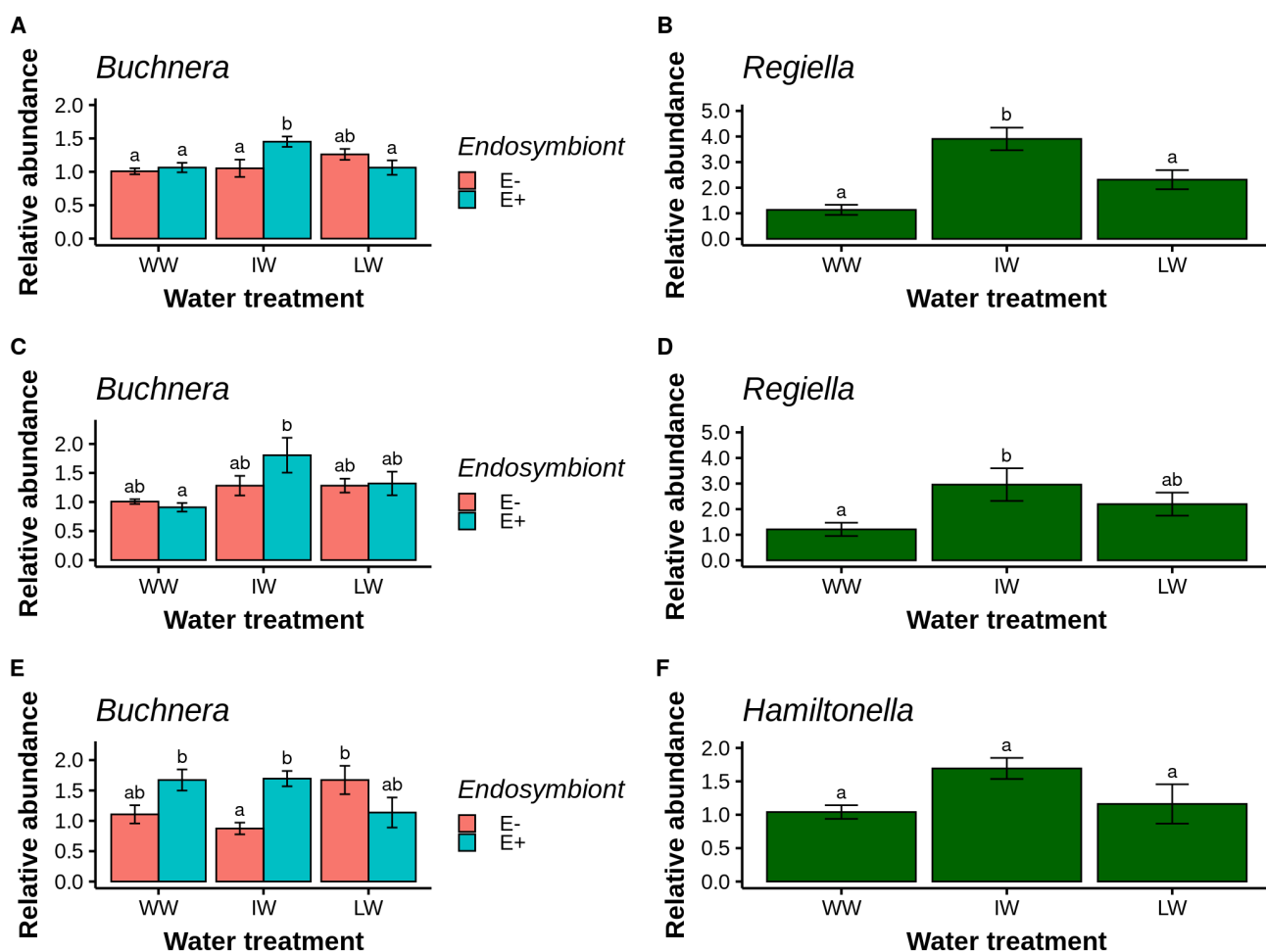


Figure 4. Relative abundance of endosymbionts in different genotypes and water treatments. *Buchnera aphidicola* (A, C, and E), *Regiella insecticola* (B and D), and *Hamiltonella defensa* (F), for genotype Sa2 (A and B), Sa3 (C and D) and SaF16 (E and F). WW, well-watered (control); IW, intermediate watering; LW, low watering. Different letters over the bars indicate significant differences ($P < 0.05$).

4 DISCUSSION

4.1 Aphids seem to overtake the effects of water deficit on their host plant

Our study observed that superclones (Sa2 and Sa3) exhibited higher reproductive performance measures as r_m , indicating a

faster population growth than the non-superclone SaF16. This helps explain the high prevalence of these genotypes in Chile, constituting over 50% of the total genotypic diversity in the region (Correa *et al.* in prep).⁴⁴ Similar r_m values have been previously documented for Chilean *S. avenae* superclones.^{47,69}

Conversely, the water treatment had varying impacts on aphid performance. The aphids' reproduction rate (r_m) was only affected by the water treatment in interaction with the endosymbiont composition, with aphids from IW (E+) showing a lower r_m than IW (E-). The main effect on r_m was observed based on the aphid genotype and its interaction with the endosymbiont composition, being significantly lower for genotype SaF16 infected individuals (E+). The effect on aphids' performance with different endosymbiont compositions in response to water deficit has not been fully explored, but it will be discussed in the following section. Conversely, the water treatment significantly impacted aphid weight in adulthood in both generations studied, particularly in the IW treatment. The aphid weight was also lower for genotype SaF16 (E+) individuals. Lastly, the water treatment did not affect the time it took for aphids to reach adulthood.

Contrasting effects have been found on insect performance under water-stressed plants. Some studies have reported a reduced performance when arthropod pests feed on drought-stressed plants.^{50,70,71} However, positive effects of water deficit on the tomato russet mite *Aculops lycopersici* have been observed, while null effects have been reported in the cases of the corn earworm *Heliothis zea* and the beet armyworm *Spodoptera exigua*.^{72,73} Interestingly, our results indicate that the intermediate water treatment positively affects aphid weight. Still, the r_m was only affected by water treatment in combination with the endosymbiont composition and only in IW. Previous studies have also reported that mild stress in wheat plants seems beneficial for *S. avenae*, probably due to a higher concentration of amino acids and sugars in sap without a significant loss of turgor.^{74,75} Under severe drought conditions, wheat has exhibited increased levels of free amino acids, including alanine, aspartic acid, glutamic acid, glutamine, glycine, serine, threonine, tyrosine, and valine, compared with well-watered plants.⁷⁶ Curiously, this high concentration of amino acids caused a poor performance in the cereal aphid *Rhopalosiphum padi* (Hemiptera: Aphididae).⁷⁶ The contrasting effect between the increased levels of free amino acids and *R. padi* was attributed to the high glutamic acid content found in drought-stressed plants, which has been associated with reduced nutritional quality and inhibition of aphid feeding.⁷⁷ Moreover, drought stress negatively impacted plant vigor by increasing plant concentrations of defensive chemicals, which suggests that the observed response of aphids is associated with reduced plant vigor and increased chemical defenses in drought-stressed plants.^{70,78,79}

4.2 The impact of endosymbionts in aphids reared under water deficit

Insects regularly face diverse forms of biotic and abiotic stressors. In response, insects activate molecular pathways that might influence their microbiome.⁸⁰ Moreover, endosymbionts that share a symbiotic bond with their hosts frequently employ common signaling pathways.⁸¹

Our research shows a significant difference in the levels of the obligate endosymbiont, *B. aphidicola*, and facultative endosymbionts between the genotypes studied under regular watering (control) conditions (WW). Specifically, genotype Sa2 had a higher abundance of *B. aphidicola* than Sa3, and a similar trend was observed for the abundance of *R. insecticola*. A specific relationship between the interaction genotype and endosymbiont can explain the difference in the abundance of endosymbionts between Sa2 and Sa3. This relationship has also been observed in the pea aphid, where a higher abundance of endosymbionts

was not associated with fitness.⁸² Our findings indicated no significant differences in *B. aphidicola* abundance between antibiotic-treated and non-treated individuals from the studied genotypes under control watering (WW). This result confirms that the combination of antibiotics used to eliminate facultative endosymbionts did not affect the abundance of the obligate endosymbiont.⁸³

Recent research has reported that the host immune system plays a critical role in regulating the abundance of endosymbionts.⁸⁴ Specifically, *R. insecticola* has been found to suppress the immune system of specific pea aphid genotypes and increase its abundance.⁸⁴ The higher abundance of *R. insecticola* found in superclones under IW can be explained, in part, by an increase in nutrients in wheat plants due to mild water stress, as has been previously reported.⁷⁶ Furthermore, the high frequency of *R. insecticola* found in *S. avenae* in Chile,³¹ seems to suggest that *R. insecticola* has no or limited fitness costs at the field level. Interestingly, *R. insecticola* appears to 'cooperate' with *B. aphidicola* to exploit resources found in wheat plants under IW. The obligate endosymbiont *B. aphidicola* may be crucial in how aphids respond to drought stress. Indeed, *B. aphidicola* can over-express essential enzymes, such as phospho-2-dehydro-3-deoxyheptonate aldolase, which is involved in the metabolism of essential amino acids and proteins that can compensate for the effects of water deficit as observed in the peach-potato aphid *Myzus persicae*.⁸⁵ Regrettably, no further studies have been conducted to examine the internal interaction between obligate and facultative endosymbionts under water deficit conditions. Therefore, additional research is required to confirm whether the increased abundance of *B. aphidicola* and *R. insecticola* under water deficit is result of 'cooperation' between them and the mechanism that controls the abundance of endosymbionts in aphids under water deficit.

On the other hand, the presence of the facultative endosymbiont *H. defensa* in genotype SaF16, a bacterium well-known for conferring protection against parasitoids such as in the pea aphid *Acyrtosiphon pisum*,^{21,86} showed adverse effects on the aphid performance regardless the watering treatment. It is worth noting that infections with endosymbionts like *H. defensa* can involve trade-offs. For instance, aphids carrying *H. defensa* have been observed to display resistance to parasitoids but also exhibit reduced behavioral defenses such as dropping behavior in the pea aphid,⁸⁷ decreased longevity in the black bean aphid *Aphis fabae*,⁸⁸ and diminished survival rate, offspring, and longevity in the corn leaf aphid *Rhopalosiphum maidis*.⁵⁵ Hence, our results confirm the occurrence of fitness cost associated with *H. defensa*.

Similar to Sa2 and Sa3, genotype SaF16 (E+) showed a higher abundance of *B. aphidicola* in both WW and IW treatments than cured individuals. Conversely, SaF16 displayed a marginally significant increase in the abundance of *H. defensa* under LW, which suggests that when resources are limited, *H. defensa* may become more abundant at the expense of *B. aphidicola*. However, definitive conclusions cannot be drawn due to the limited research on endosymbionts and their involvement in aphid responses to water deficit.

4.3 Consequences of water deficit for the pest integrated management

The impact of water stress on multitrophic interactions is not fully understood, especially in the context of biological control of insect pests by parasitoid wasps. Climate change poses a significant threat to the efficacy of biological control, particularly regarding parasitoid wasps, whose sensitivity to temperature

and water availability can lead to outbreaks of insect pests,^{89–92} disrupting the essential synchronicity between aphids and parasitoid wasps.⁹³ Our results indicate that wheat plants under a moderate water deficit (IW) produce bigger aphids, which could mean that aphids feed more and, hence, can cause greater damage to plants. Furthermore, the water deficit has been observed to have an impact on the natural enemies of aphids, causing bottom-up effects,^{94–96} including changes in aphid-natural enemy interactions, shifts in predator preferences and abundances,⁹⁷ alterations in the search and recognition cues of biological control agents,^{98–100} and a decrease in survival during the pupal stage of the parasitoid wasp *Aphidius ervi*,¹⁰¹ which serves as the primary biocontrol agent for *S. avenae* in Chile.¹⁰²

Our research demonstrates that *S. avenae* aphid superclones exhibit tolerance to water deficits, raising concerns due to the ongoing severe drought in central Chile.¹⁰³ This situation may impede the effectivity of biological control of *S. avenae* populations by their natural enemies, suggesting the potential need for regular insecticide applications, which could adversely affect the agroecosystem health.

5 CONCLUSIONS

Ensuring food security is critical in the face of climate change. Studying essential players in the functioning of agroecosystems, such as endosymbionts in insect pests and their impact on insect performance, could help anticipate pest outbreaks and create preemptive sustainable practices to deal with the effects of water deficit and climate change. Our study provides valuable insights into the complex interactions between aphid genotypes, endosymbionts, and their response to water deficit. These findings suggest that the distinct internal interactions between aphid genotypes and their endosymbionts may play a significant role in the invasivity and success of aphids facing climate change effects, even though those interactions do not affect the studied traits. Our findings also suggest that *S. avenae* aphid superclones show significant resilience to water deficit, which is worrying given the current severe drought in Chile and the world.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

- Wiebe K, Lotze-Campen H, Sands R, Tabeau A, van der Mensbrugge D, Biewald A *et al.*, Climate change impacts on agriculture in 2050 under a range of plausible socioeconomic and emissions scenarios. *Environ Res Lett* **10**:85010 (2015).
- Mirzabaev A, Kerr RB, Hasegawa T, Pradhan P, Wreford A, Tirado Von Der Pahlen MC *et al.*, Severe climate change risks to food security and nutrition. *Clim Risk Manag* **39**:100473 (2022).
- He X, Estes L, Konar M, Tian D, Anghileri D, Baylis K *et al.*, Integrated approaches to understanding and reducing drought impact on food security across scales. *Curr Opin Environ Sustain* **40**:43–54 (2019).
- FAO, *The impacts of disasters and crisis in agriculture and food security*. FAO, Rome, pp. 1–143 (2018).
- Shavit R, Batyrshina ZS, Dotan N and Tzin V, Cereal aphids differentially affect benzoxazinoid levels in durum wheat. *PLoS One* **13**:e0208103 (2018).
- Sun Y, Guo H and Ge F, Plant–aphid interactions under elevated CO₂: some cues from aphid feeding behavior. *Front Plant Sci* **7**:502 (2016).
- Rezaei EE, Siebert S, Hüging H and Ewert F, Climate change effect on wheat phenology depends on cultivar change. *Sci Rep* **8**:4891 (2018).
- Yang X, Lu M, Wang Y, Wang Y, Liu Z and Chen S, Response mechanism of plants to drought stress. *Horticulturae* **7**:50 (2021).
- Lambers H, Chapin FSI and Pons TL, *Plant Physiological Ecology*. Springer, Second edition (2008).
- Tooker JF, O'Neal ME and Rodriguez-Saona C, Balancing disturbance and conservation in agroecosystems to improve biological control. *Annu Rev Entomol* **65**:81–100 (2020).
- Bebber DP, Ramotowski MAT and Gurr SJ, Crop pests and pathogens move polewards in a warming world. *Nat Clim Chang* **3**:985–988 (2013).
- Macfadyen S, McDonald G and Hill MP, From species distributions to climate change adaptation: knowledge gaps in managing invertebrate pests in broad-acre grain crops. *Agric Ecosyst Environ* **253**:208–219 (2018).
- Furlong MJ and Zalucki MP, Climate change and biological control: the consequences of increasing temperatures on host–parasitoid interactions. *Curr Opin Insect Sci* **20**:39–44 (2017).
- Lamichhane JR, Barzman M, Booij K, Boonekamp P, Desneux N, Huber L *et al.*, Robust cropping systems to tackle pests under climate change. A review. *Agron Sustain Dev* **35**:443–459 (2015).
- Simon JC and Peccoud J, Rapid evolution of aphid pests in agricultural environments. *Curr Opin Insect Sci* **26**:17–24 (2018).
- White JA, Giorgini M, Strand MR and Pennacchio F, Arthropod endosymbiosis and evolution, in *Arthropod Biology and Evolution*, ed. by Minelli A, Boxshall G and Fusco G. Springer, Heidelberg (2013).
- Douglas AE, Nutritional interactions in insect-microbial symbioses: aphids and their symbiotic bacteria Buchnera. *Annu Rev Entomol* **43**:17–37 (1998).
- Castañeda LE, Sandrock C and Vorburger C, Variation and covariation of life history traits in aphids are related to infection with the facultative bacterial endosymbiont *Hamiltonella defensa*. *Biol J Linn Soc* **100**:237–247 (2010).
- Luo C, Monticelli LS, Li D, Suhail Ahmed S, Pandharikar G, Zhao H *et al.*, Comparison of life-history traits and resistance for *Sitobion avenae* (Fabricius) harboring a facultative symbiont. *Entomol Gen* **40**:39–47 (2020).
- Oliver KM, Degnan PH, Burke GR and Moran NA, Facultative symbionts in aphids and the horizontal transfer of ecologically important traits. *Annu Rev Entomol* **55**:247–266 (2010).
- Vorburger C, Symbiont-conferred resistance to parasitoids in aphids—challenges for biological control. *Biol Control* **116**:17–26 (2018).
- Montllor CB, Maxmen A and Purcell AH, Facultative bacterial endosymbionts benefit pea aphids *Acyrtosiphon pisum* under heat stress. *Ecol Entomol* **27**:189–195 (2002).
- Tsuchida T, Koga R and Fukatsu T, Host plant specialization governed by facultative symbiont. *Science* (1979) **303**:1989 (2004).

- 24 Dedryver CA, Le Ralec A and Fabre F, The conflicting relationships between aphids and men: a review of aphid damage and control strategies. *C R Biol* **333**:539–553 (2010).
- 25 Figueroa CC, Fuentes-Contreras E, Molina-Montenegro MA and Ramírez CC, Biological and genetic features of introduced aphid populations in agroecosystems. *Curr Opin Insect Sci* **26**:63–68 (2018).
- 26 Starý P, The Aphidiidae of Chile (hymenoptera, Ichneumonoidea, Aphidiidae). *Dtsche Entomol Z* **42**:113–138 (1995).
- 27 Apablaza J, Presencia de Macrosiphum *Sitobium avenae* (Fabricius) (Homoptera, Aphididae) en sementeras de trigo en Chile. *Cienc Investig Agrar* **1**:69–70 (1974).
- 28 Zuñiga E, Van den Boch R, Drea J and Gruber F, The biological control project against the cereal aphids (Hom., Aphididae) in Chile. II. Exploration, importation and quarantine of predator and parasitoid species. *Agricultura Técnica (Chile)* **46**:479–487 (1986).
- 29 Figueroa CC, Simon J-C, Le GJ-F, Prunier-Leterme N, Briones LM, Dedryver C-A et al., Genetic structure and clonal diversity of an introduced pest in Chile, the cereal aphid *Sitobion avenae*. *Heredity (Edinb)* **95**:24–33 (2005).
- 30 Vorburger C, Lancaster M and Sunnucks P, Environmentally related patterns of reproductive modes in the aphid *Myzus persicae* and the predominance of two “superclones” in Victoria, Australia. *Mol Ecol* **12**:3493–3504 (2003).
- 31 Sepúlveda DA, Barrueto G, Correa MCG, Castañeda LE and Figueroa CC, Spatial and temporal variation in the aphid–parasitoid interaction under different climates. *Agriculture* **11**:344 (2021).
- 32 Łukasik P, Dawid M a, Ferrari J and Godfray HCJ, The diversity and fitness effects of infection with facultative endosymbionts in the grain aphid, *Sitobion avenae*. *Oecologia* **173**:985–996 (2013).
- 33 Zytynska SE, Sturm S, Hawes C, Weisser WW and Karley A, Floral presence and flower identity alter cereal aphid endosymbiont communities on adjacent crops. *J Appl Ecol* **60**:1409–1423 (2023).
- 34 Sepúlveda DA, Zepeda-Paulo F, Ramírez CC, Lavandero B and Figueroa CC, Diversity, frequency, and geographic distribution of facultative bacterial endosymbionts in introduced aphid pests. *Insect Sci* **24**:511–521 (2017).
- 35 Ali S, Sajjad A, Shakeel Q, Farooqi MA, Aqueel MA, Tariq K et al., Influence of bacterial secondary symbionts in *Sitobion avenae* on its survival fitness against Entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium brunneum*. *Insects* **13**:1037 (2022).
- 36 Łukasik P and Kolasa MR, With a little help from my friends: the roles of microbial symbionts in insect populations and communities. *Philos Trans R Soc B* **379**:20230122 (2024).
- 37 Ma C-S, Ma G and Pincebourde S, Survive a warming climate: insect responses to extreme high temperatures. *Annu Rev Entomol* **66**:163–184 (2020).
- 38 Corbin C, Heyworth ER, Ferrari J and Hurst GDD, Heritable symbionts in a world of varying temperature. *Heredity* **118**:1–11 (2016).
- 39 Tougeron K, Iltis C, Rampoux E, Goerlinger A, Dhondt L and Hance T, Still standing: the heat protection delivered by a facultative symbiont to its aphid host is resilient to repeated thermal stress. *Curr Res Insect Sci* **3**:100061 (2023).
- 40 Heyworth ER, Smees MR and Ferrari J, Aphid facultative symbionts aid recovery of their obligate symbiont and their host after heat stress. *Front Ecol Evol* **8**:1–10 (2020).
- 41 Sunnucks P, England PR, Taylor AC and Hales DF, Microsatellite and chromosome evolution of parthenogenetic *Sitobion* aphids in Australia. *Genetics* **144**:747–756 (1996).
- 42 Wilson A, Massonnet B, Simon JC, Prunier-Leterme N, Dolatti L, Llewellyn KS et al., Cross-species amplification of microsatellite loci in aphids: assessment and application. *Mol Ecol Notes* **4**:104–109 (2004).
- 43 Peccoud J, Bonhomme J, Mahéo F, de la Huerta M, Cosson O and Simon J-C, Inheritance patterns of secondary symbionts during sexual reproduction of pea aphid biotypes. *Insect Sci* **21**:291–300 (2013).
- 44 Sepúlveda DA, ¿La variación en la composición de bacterias endosimbóticas modifica la agresividad de un insecto plaga?: consecuencias para el manejo. Universidad de Talca (Chile). Facultad de Ciencias Agrarias (2021).
- 45 Koga R, Tsuchida T, Sakurai M and Fukatsu T, Selective elimination of aphid endosymbionts: effects of antibiotic dose and host genotype, and fitness consequences. *FEMS Microbiol Ecol* **60**:229–239 (2007).
- 46 McLean A HC, van Asch M, Ferrari J and Godfray HCJ, Effects of bacterial secondary symbionts on host plant use in pea aphids. *Proc Biol Sci* **278**:760–766 (2011).
- 47 Gonzalez-Gonzalez A, Cabrera N, Rubio-Meléndez ME, Sepúlveda DA, Ceballos R, Fernández N et al., Facultative endosymbionts modulate the aphid reproductive performance on wheat cultivars differing in contents of benzoxazinoids. *Pest Manag Sci* **80**:1949–1956 (2023).
- 48 Maldonado-Ibarra I, Rodríguez GR and Castillo-Rosales D, Determination of genetic coefficients of three spring wheat varieties under a Mediterranean environment applying the DSSAT model. *Chil J Agric Res* **75**:418–424 (2015).
- 49 Matus I, Madariaga R, Jobet C, Zúñiga J and Alfaro C, Millán-INIA, new variety of high quality early spring bread wheat for irrigated soils in central-southern Chile. *Chil J Agric Res* **71**:329–332 (2011).
- 50 Dai P, Liu D and Shi X, Impacts of water deficiency on life history of *Sitobion avenae* clones from semi-arid and moist areas. *Ann Entomol Soc Am* **108**:2250–2258 (2015).
- 51 Sahoo U, Gaikwad D, Banerjee M and Maitra S, Artificial Media for Soil-less Cultivation, in *Advances in Agricultural Technology*, ed. by Maitra S, Dinkar JG and Santosh D. Griffon, Canada (2023).
- 52 Kansman JT, Basu S, Casteel CL, Crowder DW, Lee BW, Nihranz CT et al., Plant water stress reduces aphid performance: exploring mechanisms driven by water stress intensity. *Front Ecol Evol* **10**:1–11 (2022).
- 53 Wyatt IJ and White PF, Simple estimation of intrinsic increase rates for aphids and Tetranychid mites. *J Appl Ecol* **14**:757 (1977).
- 54 Leather SR, Awmack CS and Garratt MPD, Growth and development, in *Aphids as Crops Pests*, ed. by van Emden HF and Richard H. CABI, Wallingford, UK (2017).
- 55 Liu S, Liu X, Zhang T, Bai S, He K, Zhang Y et al., Secondary symbionts affect aphid fitness and the titer of primary symbiont. *Front Plant Sci* **14**:1096750 (2023).
- 56 Zhou X, Ling X, Guo H, Zhu-Salzman K, Ge F and Sun Y, *Serratia symbiotica* enhances fatty acid metabolism of pea aphid to promote host development. *Int J Mol Sci* **22**:5951 (2021).
- 57 Niu R, Zhu X, Wang L, Zhang K, Li D, Ji J et al., Evaluation of *Hamiltonella* on *Aphis gossypii* fitness based on life table parameters and RNA sequencing. *Pest Manag Sci* **79**:306–314 (2023).
- 58 Ogawa K and Miura T, Aphid polyphenisms: trans-generational developmental regulation through viviparity. *Front Physiol* **5**:73449 (2014).
- 59 Villarreal CA, González-González A, Alvarez-Baca JK, Villarreal P, Ballesteros GI, Figueroa CC, Cubillos FA, & Ramírez CC, Genome sequencing of a predominant clonal lineage of the grain aphid *Sitobion avenae*. *Insect Biochem Mol Biol* **143**:103742 (2022).
- 60 Moran NA, Russell JA, Koga R and Fukatsu T, Evolutionary relationships of three new species of Enterobacteriaceae living as symbionts of aphids and other insects. *Appl Environ Microbiol* **71**:3302–3310 (2005).
- 61 Oliver KM, Russell JA, Moran NA and Hunter MS, Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. *Proc Natl Acad Sci U S A* **100**:1803–1807 (2003).
- 62 Livak KJ and Schmittgen TD, Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* **25**:402–408 (2001).
- 63 Posit team, *RStudio: Integrated Development Environment for R*. Posit Software, PBC, Boston, MA (2024).
- 64 Spitzer JJ, A primer on Box-Cox estimation. *Rev Econ Stat* **64**:307 (1982).
- 65 Cox DR, Regression models and life-tables. *J R Stat Soc B Methodol* **34**:187–202 (1972).
- 66 Therneau TM, A Package for Survival Analysis in R. R package version 3.5-5 (2023). <https://CRAN.R-project.org/package=survival>.
- 67 Fox J and Weisberg S, *An R Companion to Applied Regression*, Third edition, Sage, Thousand Oaks (2019).
- 68 Lenth RV, Emmeans: estimated marginal means, aka least-squares means. *R package version 1.10.0* (2024). <https://CRAN.R-project.org/package=emmeans>.
- 69 Castañeda LE, Figueroa CC, Fuentes-Contreras E, Niemeyer HM and Nespolo RF, Physiological approach to explain the ecological success of “superclones” in aphids: interplay between detoxification enzymes, metabolism and fitness. *J Insect Physiol* **56**:1058–1064 (2010).
- 70 Leybourne DJ, Preedy KF, Valentine TA, Bos JIB and Karley AJ, Drought has negative consequences on aphid fitness and plant vigor: insights from a meta-analysis. *Ecol Evol* **11**:11915–11929 (2021).
- 71 Foote NE, Davis TS, Crowder DW, Bosque-Pérez NA and Eigenbrode SD, Plant water stress affects interactions between an

- invasive and a naturalized aphid species on cereal crops. *Environ Entomol* **46**:609–616 (2017).
- 72 Sconiers WB and Eubanks MD, Not all droughts are created equal? The effects of stress severity on insect herbivore abundance. *Arthropod Plant Interact* **11**:45–60 (2017).
 - 73 Ximénez-Embún MG, Glas JJ, Ortego F, Alba JM, Castañera P and Kant MR, Drought stress promotes the colonization success of a herbivorous mite that manipulates plant defenses. *Exp Appl Acarol* **73**:297–315 (2017).
 - 74 Tariq M, Wright DJ, Rossiter JT and Staley JT, Aphids in a changing world: testing the plant stress, plant vigour and pulsed stress hypotheses. *Agric Forest Entomol* **14**:177–185 (2012).
 - 75 Cui H, Wang L, Reddy GVP and Zhao Z, Mild drought facilitates the increase in wheat aphid abundance by changing host metabolism. *Ann Entomol Soc Am* **114**:79–83 (2021).
 - 76 Lin Y-Y, Liu W-C, Hsu Y-T, Hsu C-H, Hu C-C, Saska P *et al.*, Direct and knock-on effects of water stress on the nutrient contents of *Triticum aestivum* (Poales: Poaceae) and population growth of *Rhopalosiphum padi* (Hemiptera: Aphididae). *J Econ Entomol* **114**:1496–1508 (2020).
 - 77 Karley AJ, Douglas AE and Parker WE, Amino acid composition and nutritional quality of potato leaf phloem sap for aphids. *J Exp Biol* **205**:3009–3018 (2002).
 - 78 Price PW, The plant vigor hypothesis and herbivore attack. *Oikos* **62**:244–251 (1991).
 - 79 Cornelissen T, Wilson Fernandes G and Vasconcellos-Neto J, Size does matter: variation in herbivory between and within plants and the plant vigor hypothesis. *Oikos* **117**:1121–1130 (2008).
 - 80 Enders LS and Miller NJ, Stress-induced changes in abundance differ among obligate and facultative endosymbionts of the soybean aphid. *Ecol Evol* **6**:818–829 (2016).
 - 81 Hentschel U, Steinert M and Hacker J, Common molecular mechanisms of symbiosis and pathogenesis. *Trends Microbiol* **8**:226–231 (2000).
 - 82 Clavé C, Sugio A, Morlière S, Pincebourde S, Simon JC and Foray V, Physiological costs of facultative endosymbionts in aphids assessed from energy metabolism. *Funct Ecol* **36**:2580–2592 (2022).
 - 83 McLean AHC, Hrček J, Parker BJ, Mathé-Hubert H, Kaech H, Paine C *et al.*, Multiple phenotypes conferred by a single insect symbiont are independent. *Proc R Soc B* **287**:20200562 (2020).
 - 84 Nichols HL, Goldstein EB, Ziabari OS and Parker BJ, Intraspecific variation in immune gene expression and heritable symbiont density. *PLoS Pathog* **17**:e1009552 (2021).
 - 85 Verdugo JA, Sauge MH, Lacroze JP, Francis F and Ramirez CC, Drought-stress and plant resistance affect herbivore performance and proteome: the case of the green peach aphid *Myzus persicae* (Hemiptera: Aphididae). *Physiol Entomol* **40**:265–276 (2015).
 - 86 Vorburger C, The evolutionary ecology of symbiont-conferred resistance to parasitoids in aphids. *Insect Sci* **21**:251–264 (2014).
 - 87 Dion E, Polin SE, Simon J-C and Outreman Y, Symbiont infection affects aphid defensive behaviours. *Biol Lett* **7**:743–746 (2011).
 - 88 Vorburger C and Gousskov A, Only helpful when required: a longevity cost of harbouring defensive symbionts. *J Evol Biol* **24**:1611–1617 (2011).
 - 89 Torode MD, Barnett KL, Facey SL, Nielsen UN, Power SA and Johnson SN, Altered precipitation impacts on above- and below-ground grassland invertebrates: summer drought leads to outbreaks in spring. *Front Plant Sci* **7**:217942 (2016).
 - 90 Xu B, Hicke JA and Abatzoglou JT, Drought and moisture availability and recent Western spruce budworm outbreaks in the Western United States. *Forests* **10**:354 (2019).
 - 91 Harvey JA, Heinen R, Gols R and Thakur MP, Climate change-mediated temperature extremes and insects: from outbreaks to breakdowns. *Glob Chang Biol* **26**:6685–6701 (2020).
 - 92 Malina R and Praslička J, Effect of temperature on the developmental rate, longevity and parasitism of *Aphidius ervi* Haliday (Hymenoptera: Aphididae). *Plant Protect Sci* **44**:19–24 (2008).
 - 93 Welch KD and Harwood JD, Temporal dynamics of natural enemy–pest interactions in a changing environment. *Biol Control* **75**:18–27 (2014).
 - 94 Hunter MD and Price PW, Playing chutes and ladders: heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. *Ecology* **73**:724–732 (1992).
 - 95 Schoonhoven LM, Van Loon B, van Loon JJ and Dicke M, *Insect-Plant Biology*, 2nd edn. Oxford University Press, Oxford (2005).
 - 96 Han P, Bearez P, Adamowicz S, Lavoit AV, Amiens-Desneux E and Desneux N, Nitrogen and water limitations in tomato plants trigger negative bottom-up effects on the omnivorous predator *Macrolophus pygmaeus*. *J Pest Sci* (2004) **88**:685–691 (2015).
 - 97 Ahmed S, Ahmad M, Razaq M and Mahmood Shah F, Irrigation stress to wheat at sensitive growth stages: tri-trophic effects and implications for aphid control. *Pak J Zool* **54**:2815–2825 (2022).
 - 98 Boullis A, Francis F and Verheggen FJ, Climate change and tritrophic interactions: will modifications to greenhouse gas emissions increase the vulnerability of herbivorous insects to natural enemies? *Environ Entomol* **44**:277–286 (2015).
 - 99 Laws AN, Climate change effects on predator–prey interactions. *Curr Opin Insect Sci* **23**:28–34 (2017).
 - 100 Hosseini A, Hosseini M, Michaud JP, Modarres Awal M and Ghadamyari M, Nitrogen fertilization increases the nutritional quality of *Aphis gossypii* (Hemiptera: Aphididae) as prey for *Hippodamia variegata* (Coleoptera: Coccinellidae) and alters predator foraging behavior. *J Econ Entomol* **111**:2059–2068 (2018).
 - 101 Nguyen LTH, Monticelli LS, Desneux N, Metay-Merrien C, Amiens-Desneux E and Lavoit AV, Bottom-up effect of water stress on the aphid parasitoid *Aphidius ervi*. *Entomol Gen* **38**:15–27 (2018).
 - 102 Zepeda-Paulo F, Dion E, Lavandero B, Mahéo F, Outreman Y, Simon JC *et al.*, Signatures of genetic bottleneck and differentiation after the introduction of an exotic parasitoid for classical biological control. *Biol Invasions* **18**:565–581 (2016).
 - 103 Garreaud RD, Alvarez-Garretón C, Barichivich J, Boisier JP, Christie D, Galleguillos M *et al.*, The 2010–2015 megadrought in central Chile: impacts on regional hydroclimate and vegetation. *Hydrol Earth Syst Sci* **21**:6307–6327 (2017).
 - 104 Clarke HV, Genotypic and endosymbiont-mediated variation in parasitoid susceptibility and other fitness traits of the potato aphid, *Macrosiphum euphorbiae*. University of Dundee (2013).