Research Article

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Effects of fungicides on fitness and *Buchnera* endosymbiont density in *Aphis gossypii*

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Abstract

Background: Several agricultural fungicides are known to affect insect pests directly and these effects may be transgenerational and mediated through impacts on endosymbionts, providing opportunities for pest control. The cotton aphid *Aphis gossypii* is a polyphagous pest that can cause large crop yield losses. Here, we tested the effects of three fungicides, pyraclostrobin, trifloxystrobin and chlorothalonil, on the fitness and *Buchnera* endosymbiont of *A. gossypii*.

Results: The formulations of trifloxystrobin and pyraclostrobin, and the active ingredient of pyraclostrobin produced dose-dependent mortality in *A. gossypii*, whereas there was no dose-dependent mortality for chlorothalonil. The formulations of trifloxystrobin and pyraclostrobin significantly reduced the lifespan and fecundity of *A. gossypii*, and increased the density of *Buchnera* in the parental generation but not the (unexposed) F_1. When the active ingredient of pyraclostrobin was tested, the lifespan of the F_0 generation was also reduced, but the density of *Buchnera* was not, indicating that non-insecticidal chemicals in the fungicide formulation may affect the density of the endosymbiont of *A. gossypii*. There was no transgenerational effect of the active ingredient of pyraclostrobin on the lifespan and *Buchnera* of (unexposed) F_1.

Conclusions: Our results suggest that formulations of two strobilurin fungicides have immediate impacts on the fitness of *A. gossypii*, and chemicals in the formulation impact the density of the primary *Buchnera* endosymbiont. Our study highlights the potential effects of non-insecticidal chemicals of fungicides on aphid pests and their primary endosymbionts but direct connections between fitness and *Buchnera* densities remain unclear.

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

Keywords: aphid; fungicide; pesticide; endosymbiont; *Buchnera*

1 INTRODUCTION

Endosymbionts are components of the microbiome that live inside the cells of insects where they may provide essential nutrition and generate other phenotypic effects. One of the most studied examples is *Buchnera*, the primary symbiont of the pea aphid *Acyrthosiphon pisum* (and most other aphids), which provides its host with essential amino acids.[1,2] Endosymbionts in aphids and other pests may offer new targets for managing pests and the diseases they transmit.[3,4] So far, there has been some success in manipulating *Wolbachia* endosymbionts to inhibit infection and transmission of rice-ragged stunt virus by planthoppers[5] and in changing *Buchnera* endosymbiont densities in aphids to influence their resistance to avermectin.[6]

One possible avenue for targeting endosymbionts in pest control is to reconfigure applications of existing agrichemicals to reduce the density of essential endosymbionts with expected negative impacts on their hosts. Antibiotic and heat treatments can suppress endosymbionts and negatively affect host fitness.[7] Agricultural fungicides with antibiotic activity may be suitable candidates for this purpose, given that they are known to directly affect insect pests, including aphids, and these chemicals can also affect their bacterial microbiome.[8,9] In early work in this area, fungicides with potential antibiotic activity have been shown to trigger mortality and transgenerational effects in the bird cherry-oat aphid, *Rhopalosiphum padi*, but these treatments did not suppress its primary endosymbiont, *Buchnera*.[10]

The cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is an important pest that can damage crops and cause substantial economic losses worldwide. It can harm more than 300 plants from

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the families Cucurbitaceae (such as melon, marrow), Malvaceae (cotton, hibiscus, okra), Solanaceae (pepper, potato, eggplant), Rutaceae (citrus orchards) and Poaceae (wheat) by directly feeding on plant juice and spreading plant viruses.[11–13] Control of *A. gossypii* has mainly depended on chemical insecticides. *A. gossypii* has large population sizes, rapid reproduction and strong adaptability, which have led to a capacity to evolve resistance to a wide range of synthetic chemical insecticides,[14] and resistance has expanded to include organophosphates,[15] carbamates,[16] pyrethroids[17] and more recently, neonicotinoids.[18] A diverse suite of aphid management approaches beyond targeted insecticides is therefore required for long-term sustainable control.[14]

Strobilurin fungicides such as trifloxystrobin, pyraclostrobin, azoxystrobin and fluoxastrobin are considered to have high efficiency, broad-spectrum effects on fungal diseases, as well as low toxicity, limited environmental effects and other useful characteristics. However, trifloxystrobin and pyraclostrobin have a range of non-target phenotypic effects on monarch butterflies, with adult performance and wing length being reduced.[19] High concentrations of pyraclostrobin also shorten the adult lifespan of bees.[20] These fungicides can influence host microorganisms as well as the hosts themselves. Trifloxystrobin and pyraclostrobin affect spore germination and mycelial growth of *Cercospora beticola*, although chlorothalonil showed little activity against *C. beticola* in curative treatments.[21] Chlorothalonil reduces symbiotic gut bacteria in insects and increases host susceptibility to natural enemies.[22] In low-dose experiments involving two fungicides, boscalid and pyraclostrobin, bees fed the fungicide had a reduced relative abundance of bacteria *Gilliamella* sp., and the number of bacterial taxa within each bee was lower.[23] Chlorothalonil induced functional changes in the intestinal bacterial community structure of honey bees.[24] In *A. gossypii* and other aphids, azadirachtin and phoxim influence the composition of bacterial communities.[25]

In this study, the toxicity of fungicide formulations to *A. gossypii* was considered with a focus on two strobilurin fungicides (trifloxystrobin and pyraclostrobin) and the organochlorine fungicide chlorothalonil within and across generations. Although there are few studies on intergenerational biotoxicity,[26] any impact of fungicides on aphids and other pests mediated by endosymbionts may not become fully apparent until one or more generations following exposure.[27,28] Previous studies have shown that xenobiotic stress can induce changes in the abundance of *Arsenophonus* in soybean aphid,[29] and transgenerational impacts of the insecticide sulfoxaflor on *A. gossypii* may also be mediated by the endosymbionts harbored by aphids.[30] Our results suggest some fungicide formulations have immediate impacts on fitness in *A. gossypii* after exposure and the density of the primary endosymbiont *Buchnera* harbored by *A. gossypii*. However, fungicide formulations had no transgenerational impacts in this aphid on either fitness or *Buchnera* across generations.

2 MATERIALS AND METHODS

2.1 Insect and fungicides

Samples of the green-type morphs of *A. gossypii* were collected from cucumber at the Beijing Academy of Agricultural and Forestry Sciences, Haidian District, Beijing, China (116.41338°E, 39.91092°N) in November 2021. The *A. gossypii* colony was kept on cucumber in the laboratory at 18 °C, 50% relative humidity and a 16:8 h light/dark photoperiod.

To test the endosymbiont status, two replicates of five aphids per treatment were taken from the colony for molecular assessments. Genomic DNA was isolated from *A. gossypii* adults and extracted using Universal Genomic DNA Kits of Jiangsu CoWin Biotech Co., Ltd (Nanjing, Jiangsu province, China). Bacterial 16S ribosomal RNA (rRNA) genes present within DNA were amplified using the primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′)[31] with the addition of a barcode sequence and the required Illumina adapters to construct an amplicon library from the V3–V4 region of the 16S rRNA genes. Pair-end libraries with an insert size of 250 bp were sequenced on an Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA).

Sequence analysis was performed using a standard QIIME2 pipeline. Primer sequences were trimmed from reads with the cutadapt plugin. Sequence quality filtering, error correction, assembly of paired-end reads and chimera removal were performed with the DADA2 plugin. DADA2 was then used to group reads into amplicon sequence variants (ASVs), which are analogous to operational taxonomic units clustered at a 100% identity threshold. Taxonomic identity was assigned to ASVs using a naïve Bayes classifier trained against the SILVA 16S rRNA database[32] using the q2-feature-classifier plugin. The identified endosymbiont ASVs were further investigated using blastn searches against an nr/nt database. Based on sequence information, only *Buchnera* was detected in the samples; thus, further tests of the impact of treatments focused on this endosymbiont (Supporting Information, Fig. S1). The *Buchnera* ASV shared 100% sequence identity with *Buchnera* 16S ribosomal DNA (rDNA) sequences from *A. gossypii* (GenBank accession number: MK676084.1).

Formulations of 50% trifloxystrobin WG (Hebei Xingbai Agricultural Technology Co. Ltd), 250 g/L pyraclostrobin EC (Zhejiang Zhongshan Chemical Industry Group Co. Ltd) and 75% chlorothalonil WP (Pingdingshan Yinong Technology Co., Ltd) were used in this study.

2.2 Bioassay

Dose–response experiments were carried out with the two strobilurin fungicides (trifloxystrobin and pyraclostrobin) and an organochlorine fungicide (chlorothalonil) that had been tested and shown fitness effects in other work.[11] Bioassays were carried out using an aphid spray method recommended by the United Nations Food and Agriculture Organization.[33] Dose–response curves were generated using these chemicals diluted with water containing 0.1% Triton X-100 to produce five concentrations (trifloxystrobin and pyraclostrobin at 1000, 500, 250, 125 and 62.5 mg/L and chlorothalonil at 4000, 2000, 1000, 500 and 250 mg/L).

The bioassays used aphids of a similar age. To achieve this, adult aphids were placed in a Petri dish with cucumber leaves and allowed to reproduce for 24 h. Nymphs produced in these 'age-matching plates' were used for the bioassays after 4 days. Cucumber leaves that had not been exposed to pesticides were cut into disks with a diameter of 5.5 cm and put on a layer of 0.2% agar in the Petri dish. Thirty healthy nymphs were carefully transferred to each Petri dish and sprayed with chemicals using a volume of 3 mL, spray pressure of 68.9 kPa and a sedimentation time of 30 s in a Potter spray tower (Burkard Manufacturing Co. Ltd).

For the dose–response experiments, we used 80 treatment dishes (3 chemicals × 5 concentrations × 5 replicates + 5 control replicates) with 30 aphids per dish. Following exposure to each chemical/concentration, we housed aphids in an 18 °C incubator and scored mortality after 48 h. Aphids were scored as alive (moving freely), dead (no movement over 5 s) or incapacitated (unable to walk or right themselves if turned onto their back). After 48 h of fungicide exposure, we transferred all surviving aphids to a new Petri

dish and then continued to rear them for 96 h and record their mortality.

For the dose–response experiments, regression models on probits were used to examine toxicity and compute the median lethal concentration (LC_{50}) in DPS software version 12.01.[34] Relative toxicity was calculated by dividing the LC_{50} of a population by the lowest LC_{50} obtained with a fungicide. LC_{50} values were considered significantly different if their 95% confidence intervals (95% CI) did not overlap.

In a further experiment with a separate control, we tested the effect of the active ingredient of pyraclostrobin at 1000, 500, 250, 125 and 62.5 mg/L using the method described above.

2.3 Testing the effects of fungicides on *A. gossypii* life history

We used surviving aphids 96 h after exposure to the trifloxystrobin, pyraclostrobin and chlorothalonil treatments to further test for the effects of the fungicide treatments on the life history of *A. gossypii*. Trifloxystrobin and pyraclostrobin formulations had been applied at 62.5 mg/L and chlorothalonil at 4000 mg/L. A 0.1% Triton control treatment was included. Following fungicide treatment, aphids were placed in four Petri dishes with five aphids per dish; lifespan and fecundity were observed and recorded every day until all the aphids had died. This resulted in four data points per treatment for fecundity, but lifespan data were grouped across Petri fishes and therefore based on 20 individuals. To ensure that adult aphids were separated from their nymphs, newly produced F_1 nymphs were moved to separate Petri dishes every day. Exposure to the active ingredient of pyraclostrobin treatment at 62.5 mg/L was tested in a separate experiment; five aphids were individually placed in a Petri dish and observed as described above, resulting in five data points for each treatment.

Because some data were not normally distributed, differences between each treatment and the control were tested using Mann–Whitney *U* tests with Prism version 9.0.1; significance was set at the 5% level.

Survival curves of each treatment and the control were compared by Gehan–Breslow–Wilcoxon tests implemented in Prism version 9.0.1.

2.4 Testing the transgenerational effects of fungicides on *A. gossypii*

We tested the transgenerational effects of fungicide exposure by measuring the fitness and endosymbiont density of F_1 *A. gossypii*. We tested the fitness of 60 F_1 offspring for the formulations and 43 F_1 offspring for the active ingredient of pyraclostrobin by placing aphids individually in Petri dishes (note that this differs from tests with formulations at the F_0 generation in which aphids were scored in groups of five). We measured the lifespan (days alive) and fecundity (nymphs produced) of individual F_1 aphids every day until the aphids died. F_1 individuals were moved to a new Petri dish with cucumber leaves every 7 days to ensure enough food. All nymphs produced by F_1 aphids were removed and discarded.

2.5 Testing the density of endosymbiont *Buchnera* under fungicide exposure

We determined *Buchnera* density in 20 F_0 aphids after 48 and 96 h of fungicide exposure and in 20 F_1 offspring. Both sets of aphids had been age-matched and left to develop for 4 days before being preserved in 100% ethanol and stored at −20 °C for later screening. Aphids from the experiment with the active ingredient of pyraclostrobin were treated similarly.

Genomic DNA was extracted individually using a high-throughput method. Each specimen was placed in a well of a 96-well plate containing 150 μL of lysis buffer (Tris–HCl 10 mmol/L- pH 8.2, KCl 50 mmol/L, $MgCl_2$ 2.5 mmol/L, Tween-20 0.45%, gelatin 0.01%), 2 μL of proteinase K and a steel ball (2 mm in diameter)[35] and then ground with a high-throughput tissue homogenizer (MiniG 1600, SPEX SamplePreP; Metuchen, NJ, USA) for 2–5 min at 247 g. The ground specimens were incubated at 65 °C for 30 min and then at 95 °C for 10 min. The supernatant extract containing genomic DNA was kept for subsequent usage after centrifugation for 1 min at 4000 rpm. The extracted genomic DNA was quantified using a Qubit 3.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) and stored at −20 °C. The DNA was diluted tenfold. The reaction mixture (25 μL total volume) contained 12.5 μL of SYBR® Premix Ex Taq™ II (Tli RNaseH Plus) (TAKARA), 0.5 μL of ROX reference dye, 1 μL of each primer (10 μM), 5 μL of DNA, and 5 μL of RNase-free water. The following thermal cycling profile was used: 95 °C for 30 s; 40 cycles of 95 °C for 5 s and 60 °C for 34 s; 95 °C for 15 s; 60 °C for 1 min; and 95 °C for 15 s. Relative expression was calculated via the Δ*Ct* method. The quantitative polymerase chain reaction (qPCR) results are presented as the mean ± standard error for the three independent biological replicates. Expression of the target gene, the 16S rRNA gene of *Buchnera*, was determined using the primers Buch_16S_F1: AAAGCTTGCTTTCTTGTCG and Buch_16S_R1a: GGGTTCATCCAAAAGCATG[10] and the *actin* gene was used as an internal control using the primers Actin_aphid_F1: GTGATGGTGTATCTCACACTGTC and Actin_aphid_R1: AGCAGTGGTGGTGAAACTG.[36] Statistically significant differences between treatment samples and controls were determined by Mann–Whitney tests (with significance set at the 5% level, using Prism version 9.0.1).

3 RESULTS

3.1 Dose–response tests

Dose–response tests showed that pyraclostrobin exhibited greater toxicity to *A. gossypii* than trifloxystrobin, with an LC_{50} value of 38.42 mg/L compared with 156.43 mg/L for trifloxystrobin. However, an LC_{50} value for chlorothalonil could not be calculated given the absence of high mortality in any treatment (Table 1). To test whether the effect of pyraclostrobin formulations was caused by the active ingredient or adjuvant, we measured mortality in F_0 *A. gossypii* after exposure to the active ingredient of pyraclostrobin. When aphids were exposed to the active ingredient, a dose–response curve was also generated and the LC_{50} value was similar to that observed for the formulation of this chemical, with a value of 56.64 mg/L (95% CI 23.39–91.59) (Supporting Information, Fig. S2; Table 1).

3.2 Effects of fungicides on life-history traits and *Buchnera* density

Exposure to trifloxystrobin (62.5 mg/L) and pyraclostrobin (62.5 mg/L) reduced the lifespan and female offspring production of *A. gossypii* compared with the control treatment (Table 2). Lifespan differed because treated aphids start dying earlier (Fig. 1(A)). At the same time, fecundity tended to be higher in the controls than in the chemical treatments (Fig. 2(A)) and the number of nymphs produced was significantly reduced in the treatments compared with controls (Table 2). By contrast, both lifespan and offspring production in the chlorothalonil (4000 mg/L) treatment were intermediate between

controls and the other fungicides. These traits did not differ significantly from the controls (Table 2). Aphids treated with chlorothalonil tended to start dying at the same time as the other chemical treatments, but the last aphids died later than the controls (Fig. 1(A)) and continued to produce offspring (Fig. 1(A)). *Buchnera* was the only endosymbiont detected in *A. gossypii* (Supporting Information, Fig. S1). Both 48 and 96 h exposure to the three fungicides resulted in significant increases in the density of *Buchnera* compared with the control (Fig. 3(A,B)). The density of *Buchnera* did not vary consistently across time points, although higher concentrations of the fungicides led to increased *Buchnera* density at 48 h but not at 96 h.

After 96 h exposure to the active ingredient of pyraclostrobin, lifespan and number of nymphs produced per female decreased significantly in the F_0 aphids (Table 2) with patterns consistent with those obtained for the formulation (Figs 1(C) and 2(C)). However, there was no change in *Buchnera* density (Fig. 3(D)). This suggests that non-insecticidal chemicals, such as adjuvant, in the pyraclostrobin formulation rather than the active ingredient may have increased *Buchnera* density in *A. gossypii*, although the active ingredient was implicated in the fitness effects.

3.3 Transgenerational effects

Transgenerational effects of fungicide treatments on the fitness and *Buchnera* density were tested, given that these bacteria are maternally inherited. *A. gossypii* typically began producing nymphs around day 7, with a peak of nymph production occurring during days 10–20 (Fig. 2(B)). No significant difference was found between the treatments and control for survival rate (Fig. 1(B)), life-history traits (lifespan, number of nymphs produced per female, reproductive days) (Table 2; Fig. 2(B)) or *Buchnera* density (Fig. 3(C)).

For tests with the active ingredient of pyraclostrobin, the fitness of F_1 *A. gossypii*, including lifespan, number of nymphs produced per female and reproductive days did not differ from those in the control (Table 2) with similar mortality and reproduction patterns (Figs 1(D) and 2(D)). *Buchnera* density was also not affected (Fig. 3(D)), indicating that the active ingredient had no transgenerational effect on either fitness or *Buchnera* density in *A. gossypii*.

4 DISCUSSION

In this study, we tested whether three fungicides, pyraclostrobin, trifloxystrobin and chlorothalonil, with potential antibacterial properties, can impact *A. gossypii* and the endosymbiont *Buchnera* over multiple generations. Our study showed that strobilurin fungicides, including trifloxystrobin and pyraclostrobin, caused stress in aphids by shortening survival, decreasing reproduction and increasing the density of *Buchnera* in the aphids. In the case of pyraclostrobin, non-insecticidal chemicals in the formulation

Table 1. Probit analysis of the toxicity of three fungicides to *Aphis gossypii*

Fungicide	Toxicity regression ($y = ax + b$)*	LC_{50} (95% CI) (mg/L)	*P*-value
Trifloxystrobin	2.0242 + 1.3561*x*	156.43 (127.56–186.09)	0.0886
Pyraclostrobin	4.4645 + 0.3379*x*	38.42 (0.90–94.68)	0.2433
Pyraclostrobin (a.i.)	3.5685 + 0.8165*x*	56.64 (23.39–91.59)	0.7659

*Probit (y) – log(dose) (x) regression model, where a is a constant and b is the slope.
Abbreviations: a.i., active ingredient; CI, confidential interval; LC_{50}, 50% lethal concentration; *P*, probability from chi-square tests on the heterogeneity of the model.

Table 2. Effects of trifloxystrobin, pyraclostrobin and chlorothalonil formulations and active ingredient of pyraclostrobin on life table parameters of *Aphis gossypii*

Fungicide	Treatment	Lifespan (*d*)	Number of nymphs produced per female	Reproductive days (*d*)
Formulations of trifloxystrobin, pyraclostrobin and chlorothalonil	F_0-TR	16.25 ± 1.77**	2.28 ± 0.20*	NA
	F_0-PY	15.30 ± 1.43****	2.29 ± 0.20b*	NA
	F_0-CH	19.50 ± 1.73^{ns}	2.84 ± 0.28^{ns}	NA
	F_0-Control	22.40 ± 0.76	3.51 ± 0.31	NA
	F_1-TR	25.24 ± 0.86^{ns}	3.64 ± 0.47^{ns}	16.27 ± 0.65^{ns}
	F_1-PY	22.13 ± 1.06^{ns}	3.29 ± 0.46^{ns}	12.91 ± 0.79^{ns}
	F_1-CH	23.61 ± 0.99^{ns}	3.27 ± 0.43^{ns}	14.20 ± 0.84^{ns}
	F_1-Control	23.89 ± 1.08	3.67 ± 0.47	14.25 ± 0.80
Active ingredient of pyraclostrobin	F_0-PY-AI	13.20 ± 1.66*	2.49 ± 0.30*	NA
	F_0-Control	18.80 ± 0.37	5.25 ± 0.37	NA
	F_1-PY-AI	28.83 ± 1.55^{ns}	3.25 ± 14.01^{ns}	16.43 ± 0.93^{ns}
	F_1-Control	26.47 ± 1.40	3.92 ± 13.05	15.49 ± 0.86

Abbreviations: CH, chlorothalonil at 4000 mg/L; TR, trifloxystrobin at 62.5 mg/L; F_0, fungicides treatment after 96 h; F_1, fungicides treatment after next generation; NA, data not tested; PY, pyraclostrobin at 62.5 mg/L; PY-AI, active ingredient of pyraclostrobin at 62.5 mg/L.
^{ns} $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; **** $P < 0.0001$.

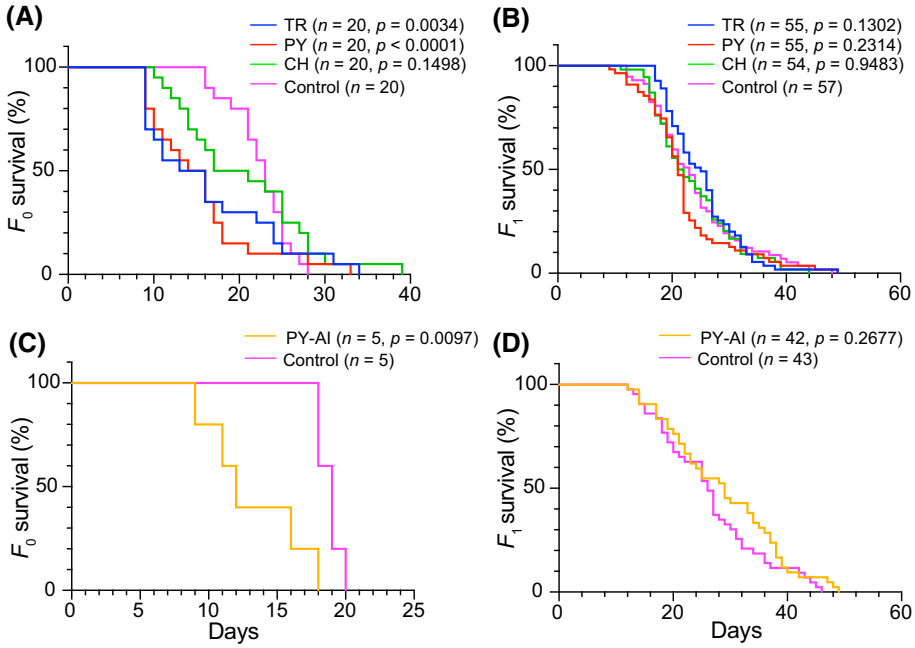

Figure 1. Survival rate of F_0 *Aphis gossypii* after 96 h (A) and the F_1 generation (B) after exposure to fungicides formulations of trifloxystrobin (62.5 mg/L), pyraclostrobin (62.5 mg/L) or chlorothalonil (4000 mg/L) as well as the control (0.1% Triton X-100). Survival rate of F_0 *A. gossypii* after 96 h (C), and the F_1 generation (D) after exposure to the active ingredient of pyraclostrobin (62.5 mg/L). Probabilities for Gehan–Breslow–Wilcoxon statistical tests comparing each treatment with the control are summarized in the figure legend, along with sample sizes. *n*, number of samples; *p*, probability. TR, trifloxystrobin at 62.5 mg/L; PY, pyraclostrobin at 62.5 mg/L; CH, chlorothalonil at 4000 mg/L; PY-AI, active ingredient of pyraclostrobin at 62.5 mg/L.

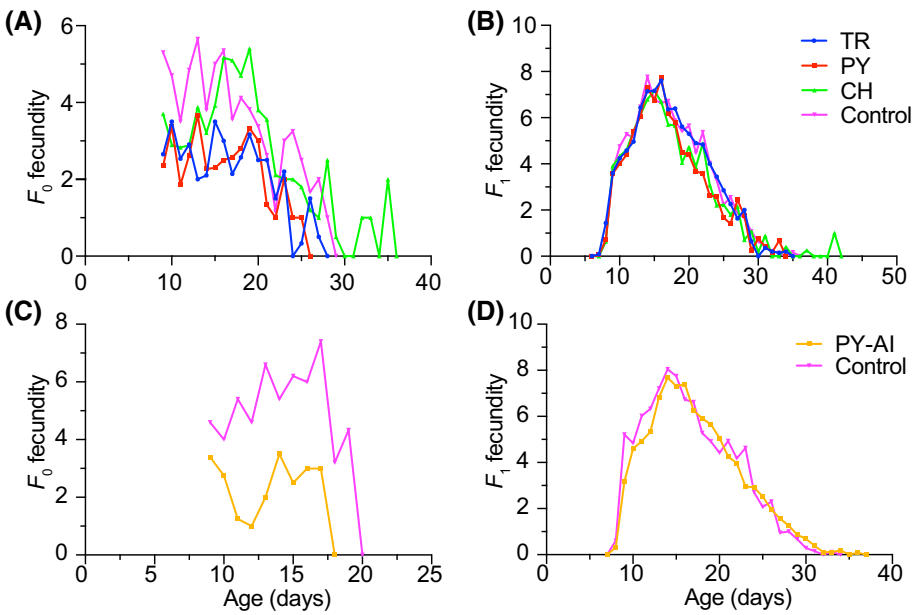

Figure 2. Fecundity of *Aphis gossypii* after treatment with fungicides. (A) Fecundity of F_0 after 96 h exposure of adults to three fungicide formulations. (B) Fecundity of F_1 after exposure of adults to three fungicide formulations. (C) Fecundity of F_0 after 96 h exposure of adults to the active ingredient of pyraclostrobin. (D) Fecundity of F_1 after exposure of adults to active ingredient of the pyraclostrobin. See Figure 1 for the abbreviation of fungicides.

may have contributed to changes in *Buchnera* density, but not the negative effects on aphid fitness. We found fitness costs of *A. gossypii* potentially caused by the active ingredients of pyraclostrobin, although the population-level effects of these fungicides remain unclear given that the aphids would have produced some offspring before the period where mortality occurred.

Strobilurins inhibit electron transfer during mitochondrial respiration in fungi[9] and are often used as fungicides in agriculture.

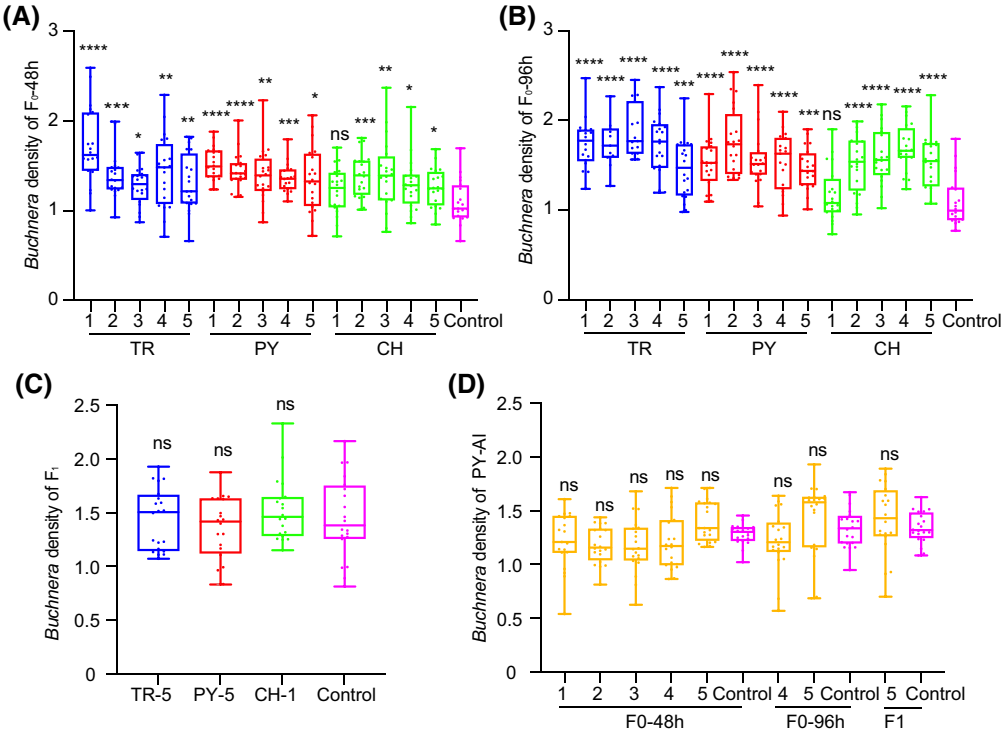

Figure 3. *Buchnera* density of F_0 *Aphis gossypii* after 48 h (A) or 96 h (B) and F_1 (C) exposure of initial adults to three fungicides formulations at five concentrations, as well as control. *Buchnera* density of F_0 after 48–96 h, F_1 exposure to the active ingredient of pyraclostrobin (D). Concentrations 1–5 of trifloxystrobin and pyraclostrobin were 1000, 500, 250, 125 and 62.5 mg/L. Concentrations 1–5 of chlorothalonil were 4000, 2000, 1000, 500 and 250 mg/L. See Figure 1 for the abbreviation of fungicides. *Significant difference; ns, no significant difference.

However, the functions of strobilurins are not limited to fungal disease control. It has been reported that strobilurin fungicides can activate plant defense against viral and bacterial infections.[37] In our study, we found that two strobilurins (at least the active ingredient of pyraclostrobin) have an insecticidal activity toward *A. gossypii*. The field-recommended dosage of trifloxystrobin and pyraclostrobin is 250 mg/L, higher than the LC_{50} concentrations toward *A. gossypii* as determined in our study. These results suggest that a regular spray of these fungicides might kill more than 50% of *A. gossypii* in the field. However, field trials are needed to test this prediction based on bioassay data and to assess population-level effects. A wide range of tests of fungicides with different mode of actions toward insect pests may help to develop integrated strategies for invertebrate pests and plant disease.

Strobilurins can also affect the life history of insects. Strobilurins can reduce wing length in monarch butterflies[19] and the adult lifespan of bees.[20] A recent study showed that pyraclostrobin and trifloxystrobin reduced the lifespan and fecundity of the aphid *R. padi* F_1.[10] Our study found that pyraclostrobin could also affect the life-history traits of *A. gossypii*, indicating that these effects of strobilurin seem to extend to aphids more generally. However, unlike in *R. padi*, where these chemicals were tested at a similar rate, neither pyraclostrobin nor trifloxystrobin affected fitness in the F_1 generation in the current experiments, suggesting that effects on aphids vary across species. Pesticide efficacy in controlling aphids and other pests is typically evaluated within the single generation during which pesticide exposure occurs.[38] Transgenerational impacts of pesticides may provide farmers with greater pest management options,[39] but current results suggest that transgenerational control will depend on the target aphid species. The effects of other fungicides such as the organochlorine chlorothalonil also seem to vary across species, given that a formulation of chlorothalonil substantially reduced the lifespan and fecundity of F_1 *R. padi*,[10] whereas no intergenerational effects were detected in our study.

The density of *Buchnera* in *A. gossypii* increased after exposure to all three fungicide formulations at different time points and concentrations, but not after exposure to the active ingredient of pyraclostrobin. Our results indicate that non-insecticidal chemicals, such as adjuvants, may alter the impact of pesticides, including strobilurin.[40,41] Non-insecticidal chemicals in fungicide formulations perhaps create stressful conditions independent of the active ingredients of the strobilurins, which then increase the density of *Buchnera*. Besides providing essential nutrition to aphids, *Buchnera* has some non-nutritive functions involving responses to environmental stresses.[42] Although the density of *Buchnera* is usually relatively stable in aphids, some studies have indicated interactions between this endosymbiont and chemical toxins affecting its density in aphids, including interactions with cry toxins,[43] sulfoxaflor[30] and avermectin.[6] A reduction in *Buchnera* may be linked to decreased host fitness under chemical pressures, such as increased susceptibility to imidacloprid following avermectin exposure.[6] A lower *Buchnera* density may also decrease the capacity to synthesize vitamins, which are essential nutrients for aphids.[1] The nutritional role of the primary (obligate) symbiont *Buchnera* for its hosts has been well established in aphids.[1]

However, an increased density of *Buchnera* as well as a decreased density may have fitness costs, such as through

competition for nutrients or the accumulation of damage in aphids. We found that exposure to pyraclostrobin generated a fitness cost together with an increase in *Buchnera* density in the F_0 generation. Perhaps there are fitness costs when the balance between the host and *Buchnera* density is disrupted. Further studies are needed to explore such effects and to disentangle the specific effects of chemical constituents of formulations on aphids.

Because of the absence of secondary endosymbionts in our aphid culture, we were not able to investigate interactions between fungicide treatments and other microbes, even though these are abundant in many aphid species, including *A. gossypii*.[30,44] Secondary symbiotic bacteria have various functions that can affect host insects. Some of these include participating in the host insect defense against natural enemies,[45,46] resisting host infection by pathogenic agents,[47] improving host heat resistance,[48] assisting virus transmission,[49] regulating host reproduction and improving host adaptability. The role of many secondary symbiotic bacteria and their interaction with chemicals remains unclear. However, in one study on the effects of sulfoxaflor on the symbiotic bacterial community of *A. gossypii* across three generations, there was a decrease in diversity following pesticide treatment and changes in the relative abundance of another endosymbiont, *Arsenophonus*.[30] It will be interesting to further investigate the effects of fungicides on secondary endosymbionts of insect pests and their related fitness effects.

5 CONCLUSIONS

Our study explored the effects of fungicides on the survival and life-history traits of *A. gossypii* and possible interactions with symbiotic bacteria. We found that strobilurin fungicides have immediate impacts on fitness in *A. gossypii* after exposure, although these do not extend to the F_1 generation as documented for another aphid species. The density of the primary endosymbiont *Buchnera* harbored by *A. gossypii* was increased by exposure to fungicides, including the organochlorine chlorothalonil that did not influence aphid fitness. However, the *Buchnera* effects may be mediated by non-insecticidal chemicals rather than the active ingredient of these fungicides. Our study identifies the potential effects of non-insecticidal chemicals on the occurrence and control of insect pests, but more research should target interactions with secondary endosymbionts.

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AUTHOR CONTRIBUTIONS

Qiong Yang, Shu-Jun Wei and Ary A. Hoffmann contributed to the conception and design of the study. Yong-Fu Gao, Ya-Jing Ren, Jin-Cui Chen, Li-Jun Cao and Guang-Hang Qiao conducted the experiments. Yong-Fu Gao and Qiong Yang analyzed the data and wrote the first draft of the manuscript. Shu-Jun Wei and Ary A. Hoffmann revised the manuscript. Shu-Jun Wei, Shi-Xiang Zong and Ary A. Hoffmann contributed to the interpretation and analysis of the data. All authors read and approved the manuscript.

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.

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