Journal of Experimental Botany, Vol. 00, No. 0 pp. 1-12, 2025
https://doi.org/10.1093/jxb/eraf208 Advance Access Publication 21 May 2025

RESEARCH PAPER

Interference with phytohormone signaling by whiteflies differentially affects plant attractiveness to a larval and an egg parasitoid of the cabbage white butterfly

Yu-Mei Dong[1], Ya-Li Sang[1], Shu-Zhen Wang[1], Ted C.J. Turlings[1,2,3,4], Ye-Hua Li[5,*], Da-Wei Xue[1,*], and Peng-Jun Zhang[1,*]

[1] College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou, China
[2] State Key Laboratory of Cotton Bio-Breeding and Integrated Utilization, School of Life Sciences, Henan University, Kaifeng 475004, China
[3] Laboratory of Fundamental and Applied Research in Chemical Ecology (FARCE), Institute of Biology, University of Neuchâtel, Switzerland
[4] Department of Entomology, The Pennsylvania State University, University Park, PA, USA
[5] Research Institute of Leisure Agriculture, Jiangsu Academy of Agricultural Sciences, Nanjing, China

* Correspondence: yehua860101@126.com, dwxue@hznu.edu.cn, or pengjun-zhang@hznu.edu.cn

Received 25 November 2024; Editorial decision 7 May 2025; Accepted 19 May 2025

Editor: Victor Flors, Universitat Jaume I, Spain

Abstract

In response to egg deposition or feeding by insect herbivores, plants release specific blends of volatiles that attract natural enemies of the herbivores. In nature, plants are often simultaneously attacked by multiple herbivores that may induce different signaling pathways, thus affecting the volatile blends and interfering with the attraction of natural enemies. The mechanisms underlying such interference remain largely unknown. Here, we show that co-infestation of *Arabidopsis thaliana* plants with the whitefly *Bemisia tabaci* reduces the volatile emissions induced by *Pieris rapae* caterpillars, resulting in reduced attraction of the larval parasitoid *Cotesia rubecula*. Hormone and gene expression analyses, followed by assays with various Arabidopsis mutants, revealed that this interference by *B. tabaci* is the result of antagonistic crosstalk between jasmonic acid and salicylic acid, involving the transcription factor NPR1 but not WRKY70. In contrast, *B. tabaci* co-infestation had no impact on the attraction of an egg parasitoid to egg-induced volatiles of *P. rapae*. These differential effects of the whitefly on the behavior of the two parasitoids were confirmed in greenhouse assays. This study provides new insight into the molecular mechanisms underlying the recruitment of different parasitoids by plants and could help in developing effective biocontrol strategies.

Keywords: *Bemisia tabaci*, multiple herbivores, parasitoid, *Pieris rapae*, plant volatiles.

Introduction

Plant volatiles play an important role in mediating the interaction between herbivorous insects and parasitic wasps (Dicke and Sabelis, 1988; Turlings *et al.*, 1990; Steinberg *et al.*, 1993; Clavijo McCormick *et al.*, 2012). In response to feeding or egg deposition by herbivorous arthropods, plants emit various blends of volatiles that differ in both quantity and composition from those emitted by uninfested plants (Dicke and Baldwin, 2010; Hilker and Fatouros, 2015). Herbivore enemies, predators and parasitoids, exploit these specific volatile blends as cues for prey and host location

(Kessler and Baldwin, 2001; Turlings and Erb, 2018; Chen *et al.*, 2021). The distinctive volatile profiles induced by various herbivores can be caused by specific elicitors in oral secretions of herbivores (Schmelz, 2015; Ling *et al.*, 2021) and the differential induction of defense signaling pathways by herbivory (Kessler and Baldwin, 2002; Pieterse and Dicke, 2007). Thus far, herbivore-induced plant volatile emissions have mostly been investigated in the context of an interaction between plants and a single herbivore species (Dicke *et al.*, 2009). However, in nature, plants are usually attacked by multiple herbivores, either simultaneously or sequentially, in shoots and roots, which might interfere with the specificity of volatile blends and thereby with natural enemy attraction (Bezemer and van Dam, 2005; Rodriguez-Saona, *et al.*, 2005; Rasmann and Turlings, 2007; Zhang *et al.*, 2009).

The jasmonic acid (JA) signal transduction pathway is commonly implicated in the induction of herbivore-induced plant volatiles (Ament *et al.*, 2004; Kessler *et al.*, 2004), although the salicylic acid (SA) and ethylene (ET) pathways are also involved in certain cases (Ruther and Kleier, 2005; Zhang *et al*, 2013a). It is increasingly apparent that different herbivores induce different signaling transduction pathways. Chewing herbivores such as caterpillars and beetles predominantly activate JA-dependent responses, as do necrotrophic pathogens, whereas phloem-feeding insects such as whiteflies and aphids mainly activate SA-dependent responses, similar to biotrophic pathogens (Kempema *et al.*, 2007; Spoel *et al.*, 2007; Howe and Jander, 2008; Zhang *et al.*, 2017; Erb and Reymond, 2019). Ample evidence indicates an antagonistic interaction between JA and SA signaling pathways (Koornneef and Pieterse, 2008; Pieterse *et al.*, 2012; Thaler *et al.*, 2012), although synergistic interactions have also been reported (van Wees *et al.*, 2000). As a result of crosstalk between SA and JA, herbivores that induce SA signaling are assumed to attenuate or otherwise affect the volatile emissions from plants infested by herbivores that induce JA signaling, thereby influencing indirect plant defenses (Dicke *et al.*, 2009). Some evidence, although rare, seems to support this assumption. For example, cotton plants infested by *Spodoptera exigua* caterpillars and whiteflies emit lower amounts of volatiles than plants infested by only caterpillars (Rodriguez-Saona *et al.*, 2005). Simultaneous infestation of rice plants by the brown planthopper and the rice striped stem borer alters plant volatile emissions in ways such that it reduces the attractiveness of the plant to egg parasitoids of the two pests (Hu *et al.*, 2020; Liu *et al.*, 2021). In contrast, in pepper, simultaneous infestation by the aphid *Myzus persicae* and spider mite *Tetranychus urticae* resulted in an enhanced emission of volatiles compared with plants infested by spider mites alone, and consequently caused a stronger attraction of a predatory mirid bug to dual-infested plants (Moayeri *et al.*, 2007). These contrasting findings highlight the complex and context-dependent nature of plant responses to simultaneous herbivory, where the interplay among the responses triggered by different herbivore species may either suppress or amplify plant volatile emissions (Cusumano *et al.*, 2015; Ponzio *et al.*, 2016). Despite various intriguing studies, a critical knowledge gap persists in understanding how different herbivore-induced signaling pathways, such as SA and JA, interact to modulate plant volatile emissions and, by extension, affect indirect defense mechanisms. It is evident that, to fully understand and predict the effects of multiple attacks on plant volatile emissions and indirect plant defense, it is important to acquire detailed knowledge on the kinetics of the induction process and the respective effects of different signaling pathways. Addressing this knowledge gap is essential for developing more effective and predictive models of plant defense, with implications for improving pest management strategies.

In the present study, we employed a model system comprising *Arabidopsis thaliana*, the small cabbage white butterfly *Pieris rapae* L. (Lepidoptera: Pieridae), and its egg parasitoid *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae), as well as the larval parasitoid *Cotesia rubecula* (Hymenoptera: Braconidae) to investigate how co-infestation with the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) affects the attraction of these two parasitoids to volatiles emitted by infested plants. We chose Arabidopsis because it is an excellent genetic model (van Poecke and Dicke, 2002; Zhang *et al.*, 2013b) that allowed us to study molecular details of the signaling pathways involved. Both herbivores are important pests of crucifers; the whitefly *B. tabaci* is a phloem-feeding insect that predominantly induces SA-dependent responses in its host plants (Zarate *et al.*, 2007), whereas *P. rapae* caterpillars are chewing insects that mainly induce JA-dependent responses (De Vos *et al.*, 2006). Importantly, recent studies found that egg deposition by *P. rapae* induces the expression of several SA-dependent genes in Arabidopsis (Little *et al.*, 2007) and that egg-induced plant volatiles are attractive to *Trichogramma* wasps (Pashalidou *et al.*, 2010; Fatouros *et al.*, 2014). Given the crosstalk between JA and SA signaling pathways and the nature of the response of Arabidopsis to *P. rapae* egg deposition and caterpillar feeding, we hypothesized that co-infestation by the whitefly will (i) interfere with the specificity of caterpillar-induced volatile blends for *C. rubecula* attraction; and (ii) increase the intensity of egg-induced volatile emission and possibly enhance *T. chilonis* attraction or at least not impair it. We tested these hypotheses by analyzing the volatile profiles of plants subjected to *P. rapae* egg deposition and caterpillar feeding, both with and without *B. tabaci* co-infestation, and then measured the behavioral responses of *C. rubecula* and *T. chilonis* to the emitted volatiles. Subsequent phytohormone and gene expression analyses were performed to pinpoint the molecular mechanism involved in the observed changes in odor emissions. We found that *B. tabaci* co-infestation indeed interfered with the volatile emissions induced by *P. rapae* caterpillars and the attraction of *C. rubecula*, and we could attribute this to the induction of SA in an NPR1-dependent manner. In contrast, *B. tabaci* co-infestation had no effect on the release of volatiles induced by *P. rapae* eggs, as was also evident from the

unaffected attraction of its egg parasitoid *T. chilonis*. The outcome of the laboratory experiments was further substantiated under greenhouse conditions in which whitefly co-infestation resulted in fewer larval parasitoids recruited by caterpillar-infested Chinese cabbage, but had no impact on the recruitment of egg parasitoids by plants that were co-infested with *P. rapae* eggs.

Materials and methods

Plants and insects

Arabidopsis thaliana ecotype Columbia-0 (Col-0; wild-type) and mutants (*npr-1*, *NahG*, and *wrky70*) were grown as described previously (Reymond *et al*., 2000). In brief, plants were grown from seed in a climate room (22 ± 1 °C, relative humidity 60–70%, 8 h light:16 h dark) under a light intensity of ~50 μmol m^{-2} s^{-1}. We used 6- to 8 week-old plants in the experiments. The mutant lines were all in the Col-0 background. White cabbage *Brassica oleracea* plants (var. *capitata* cv. Jing-feng No. 1) were grown from seed in a greenhouse (25 ± 2 °C, relative humidity 50–70%, 14 h light:10 h dark) and used in greenhouse experiments when they were 5–6 weeks old.

All insects were collected in the surroundings of Hangzhou, China. A colony of virus-free whitefly *B. tabaci* (Gennadius) MEAM1 was maintained on *B. oleracea* plants in a separate climate room (22 ± 1 °C, relative humidity 60–70%, 8 h light:16 h dark). The small cabbage white butterfly *Pieris rapae* was reared on *B. oleracea* plants in a greenhouse compartment (25 ± 2 °C, relative humidity 50–70%, 14 h light:10 h dark). The larval parasitoid *C. rubecula* was reared using *P. rapae* caterpillars that had been feeding on *B. oleracea* plants in a greenhouse. The egg parasitoid *T. chilonis* was reared using *P. rapae* eggs in a climate room (22 ± 1 °C, relative humidity 60–70%, 8 h light:16 h dark). Only mated, 3- to-5-day-old female wasps were used in the experiments.

Plant treatments

In preliminary experiments, we compared the effects of egg or caterpillar density on the attraction of *T. chilonis* or *C. rubecula* wasps to host-infested plants. At a density of 30 or 50 eggs per plant, *T. chilonis* wasps preferred the volatiles from egg-infested plants over those of uninfested plants (Supplementary Fig. S1A). At a density of three or five caterpillars per plant, *C. rubecula* wasps preferred the volatiles from caterpillar-infested plants over uninfested plants (Supplementary Fig. S1B). Overall, these data show that plants infested with higher numbers of *P. rapae* eggs or caterpillars are more attractive to parasitoid wasps. We, therefore, used the densities of 50 eggs per plant or five caterpillars per plant for subsequent bioassays. For all treatments, three densities of *B. tabaci* were tested, namely 50, 75, and 100 adults per plant. For these experiments plants were subjected to the following treatments.

Egg infestation

Plants were placed into a ventilated cage (70 × 60 × 45 cm) with >60 *P. rapae* adults (female:male ratio 1:1) to allow deposition of eggs onto the plants. Plants were exposed for no more than 30 min to the butterflies. After this exposure time, only 50 eggs were left on the leaves of selected infested plants after removing the surplus eggs using a fine camel-hair brush. Thereafter, plants were used for experiments.

Caterpillar infestation

Five newly hatched *P. rapae* larvae (0–24 h old) were placed on each plant. The plants were not caged because the caterpillars do not move from plant to plant as long as the leaves of adjacent plants do not touch each other.

Egg and whitefly infestation

For each density of *B. tabaci*, plants were infested with *P. rapae* eggs and adult *B. tabaci* whiteflies in three different sequences. (i) First eggs then whiteflies: plants were initially infested with *P. rapae* eggs for 3 d. Subsequently, plants carrying the eggs were infested with adult *B. tabaci* (treatment 1) or kept free of *B. tabaci* infestation (control 1) for 3 d. (ii) First whiteflies then eggs: plants were initially infested with adult *B. tabaci* (treatment 2) or left uninfested (control 2) for 3 d. After that, *B. tabaci* whiteflies were removed from leaves, and subsequently both treated and control plants were infested with *P. rapae* eggs for 3 d. (iii) Eggs and whiteflies simultaneously: plants were infested simultaneously with *P. rapae* eggs and *B. tabaci* (treatment 3), or infested with *P. rapae* eggs only (control 3) for 3 d. Before bioassays, *B. tabaci* adults were removed from the plants by aspiration, while *P. rapae* eggs remained.

Caterpillar and whitefly infestation

For each density of *B. tabaci*, plants were infested with *P. rapae* caterpillars and adult *B. tabaci* whiteflies in three different sequences. (i) First caterpillars then whiteflies: plants were initially infested with *P. rapae* caterpillars for 3 d. Subsequently, plants carrying the caterpillars were infested with adult *B. tabaci* (treatment 4) of kept free of *B. tabaci* infestation (control 4) for 3 d. (ii) First whiteflies then caterpillars: plants were initially infested with adult *B. tabaci* (treatment 5) or left uninfested (control 5) for 3 d. After that, *B. tabaci* whiteflies were removed from leaves, and subsequently both treated and control plants were infested with *P. rapae* caterpillars for 3 d. (iii) Caterpillars and whiteflies simultaneously: plants were infested simultaneously with *P. rapae* caterpillars and *B. tabaci* (treatment 6), or infested with *P. rapae* eggs only (control 6) for 3 d. Before bioassays, *B. tabaci* adults were removed from the plants by aspiration, while *P. rapae* caterpillars remained.

Olfactometer experiments

Behavioral responses of parasitoids to plant volatiles were assessed with a Y-tube olfactometer. The transparent glass Y-tube olfactometer had a 20 cm stem and two 20 cm arms placed at a 75° angle, all with an internal diameter of 1.5 cm. A glass funnel (port diameter = 8 mm) was attached to the inner wall at the midpoint of each arm, just before a 12 mm vertical male ground-glass connector attached to a 25 ml wasp-trapping bulb. This setup was adapted for small wasps such as *Trichogramma* sp. Two streams of purified air (filtered through activated charcoal) were directed into a 2.5 liter glass container holding an odor source, with airflow leading into the olfactometer arms at 100 ml min^{-1}. All glass components were connected using Teflon tubing. The experiments were conducted between 09.00 h and 15.00 h at 23 ± 2 °C, under T5-growth lights with a spectrum approximating natural sunlight (Philips, China). Before placing a plant into the glass containers, the pot was carefully removed, and the roots and soil were covered with aluminum foil.

To begin the experiment, 10 wasps were released at the base of the Y-tube. After 30 min, the wasps trapped in the two wasp-trapping bulbs at the ends of each arm were counted. Wasps that did not make a choice within 30 min were recorded as 'no response' and excluded from statistical analysis. To avoid potential asymmetry in the setup, odor sources were interchanged after each trial. Four plants from each treatment group were used as odor sources, and the experiment was repeated during 3 d, with 20 wasps per day for each odor comparison.

Volatile collections and analyses

Volatiles emitted from Arabidopsis plants were collected using a dynamic headspace collection system in a climate chamber maintained at 25 ± 2 °C with 50–70% relative humidity. Two hours before volatile trapping, the plant pot was carefully removed, and the root and soil were covered with aluminum foil. Four plants from the same treatment group were placed

together in a 2.5 liter glass vessel. The vessel was sealed with a glass lid using a Viton® O-ring and a metal clamp to ensure an airtight seal. Purified air, filtered through silica gel, a molecular sieve, and activated charcoal, was split into two air streams, each with a constant flow of 200 ml min^{-1}, and directed into two vessels through Teflon tubing. This allowed for simultaneous volatile collection from a treatment plant and its corresponding control. The system was purged with purified air for 1 h before attaching a tube filled with Tenax TA (90 mg, Grace-Alltech, Deerfield, IL, USA) to the air outlet in the lid to trap the headspace volatiles. After 3 h of trapping, the aerial parts of four plants were weighed to standardize volatile emissions. Volatile collection for each treatment was repeated five times.

Headspace samples were analyzed using a Thermo TraceGC Ultra connected to a Thermo TraceDSQ quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Prior to thermodesorption, the traps were flushed with helium at 30 ml min^{-1} for 15 min to remove moisture and oxygen. After flushing, the collected volatiles were desorbed from the Tenax traps at 220 °C (Ultra; Markes, Llantrisant, UK) for 5 min with a helium flow of 30 ml min^{-1}. The released compounds were focused on an electrically cooled sorbent trap (Unity; Markes) at 0 °C. Volatiles were injected onto an analytical column (Rtx-5 ms, 30 m × 0.25 mm ID, 1.0 µm film thickness, Restek, Bellefonte, PA, USA) using a split flow mode of 20 ml min^{-1} by rapidly heating the cold trap to 250 °C for 3 min. The temperature program began at 40 °C (3 min hold), followed by an increase of 10 °C min^{-1} until reaching 280 °C (3 min hold). The column effluent was ionized by electron impact (EI) at 70 eV, and mass scanning was performed from 33 m/z to 250 m/z at a scan rate of 4.7 scans s^{-1}. Eluted compounds were identified using Xcalibur software (Thermo Fisher Scientific) by comparing the mass spectra with authentic reference standards or with NIST 08 and Wiley library spectra. Quantification of identified compounds was based on comparison with a set of authentic standards [including (*E*)-2-hexenal, linalool, methyl salicylate, and β-myrcene; Sigma-Aldrich] injected in five concentrations ranging from 2.5 ng μl^{-1} to 40 ng μl^{-1} methanol. Response factors for all reference compounds were linear within this concentration range.

Total RNA isolation and cDNA synthesis

Leaf tissues collected from three plants of each treatment were pooled into a single sample and immediately frozen in liquid nitrogen. Total RNA was extracted from 150 mg of each leaf sample using the RNeasy Plant Mini Kit (Qiagen, Germany). RNA concentration and purity were assessed using a NanoDrop™ Spectrophotometer ND-2000 (Thermo Scientific, Wilmington, DE, USA), and RNA integrity was verified by 1% agarose gel electrophoresis with ethidium bromide staining. For cDNA synthesis, 1 µg of total RNA was reverse-transcribed using the First-Strand cDNA Synthesis Kit (TaKaRa, Hangzhou, China) in a final reaction volume of 25 µl.

Quantitative real-time PCR

Gene-specific primers were designed to produce amplicons of 150–300 bp from the 3′ end of the cDNA strand. Amplification reactions were performed in a final volume of 20 µl, containing 2 µl of cDNA, 10 µl of iQ™ SYBR® supermix (BioRad, Hangzhou, China), and 0.8 µl of each forward and reverse primer (5 µM). Quantitative real-time PCR (qRT-PCR) analysis was carried out using the ABI 7500 Real-Time PCR System with the following cycling conditions: 95 °C for 5 min, followed by 40 cycles of 10 s at 95 °C and 20 s at 55–60 °C. A melting curve analysis was performed from 60 °C to 95 °C, with a 5 s hold at each temperature increment. Primers used for qRT-PCR are listed in Supplementary Table S1. RT-PCR analyses were done with at least three biological replicates of each sample and two technical replicates of each biological replicate. Gene expression was normalized with two reference genes, *SAND* (At2g28390) and *UBC* (At5g25760), as recommended by Czechowski *et al.* (2005). Gene expression data were analyzed by geometric averaging of the two reference genes, followed by the normalized calculation using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001; Vandesompele *et al.*, 2002).

Quantification of endogenous jasmoci acid and salicylic acid

Plant material (250–300 mg) was frozen and ground under liquid nitrogen. For quantification, [9,10-$^2H_2$]9,10-dihydro-JA (100 ng) and [3,4,5,6-$^2H_4$]SA (200 ng) were added as internal standards. JA, SA, and the internal standards were partitioned to an aqueous phase via centrifugation and vaporization. The aqueous phase was then extracted with an equal volume of ethyl acetate and subsequently dried. The dried extract was resuspended in 0.1 M acetic acid and loaded onto a C18 column (Waters Company, Milford, MA, USA). The column was eluted sequentially with solvent mixtures of acetic acid/methanol (v/v) at ratios of 83/17, 60/40, and 40/60. The effluents of the last 4 ml in 40% methanol and the first 3 ml in 60% methanol were collected. After solvent evaporation, the residue was esterified using excess ethereal diazomethane. Samples were analyzed with a Finnigan GCQ ion trap mass spectrometer (Thermoelectron, Bremen, Germany). The instrument was run in a CI-negative ion mode.

Greenhouse experiments

To determine whether whitefly feeding affects the parasitism rate of egg or larval parasitoids, we investigated the preference of wasps between plants infested by hosts and plants infested by hosts and whiteflies in a greenhouse (12 × 7 m). To prevent whiteflies from moving from dual-infested plants to egg- or caterpillar-infested plants, clip cages were used to confine whiteflies to specific leaves. However, in preliminary experiments with Arabidopsis plants, we found that the clip cages caused noticeable mechanical damage to the leaves. Since mechanical damage can affect volatile emissions from Arabidopsis (Matsui, 2006), we opted to use *B. oleracea*, a relative of Arabidopsis, for the greenhouse experiments for two primary reasons: (i) the clip cages used in the experiments do not cause significant mechanical damage to *B. oleracea* leaves, in contrast to applying them on Arabidopsis, which ensured that the observed effects are not confounded by physical injury; and (ii) *B. oleracea* serves as a natural host plant for both *B. tabaci* and *P. rapae* in the field (Zhang *et al.*, 2013), rendering it a more ecologically relevant model for studying the tri-trophic interactions under conditions that reflect those in a natural setting. In the first experiment, we compared the number of eggs parasitized by *T. chilonis* wasps when they were given a choice between plants infested with eggs only and plants infested with both eggs and whiteflies. Prior to a bioassay, *B. oleracea* plants were placed in a cage with >100 *P. rapae* adults (female:male ratio 1:1) to allow for egg deposition. Plants were exposed to the butterflies for no more than 30 min to obtain 50–80 eggs per plant. After this, one group of egg-infested plants was kept in a climate-controlled room, while another group was infested with 100 adult whiteflies, which were confined to leaves using clip cages. After 3 d of infestation, one plant infested with eggs only and one plant infested with eggs and whiteflies were placed in diagonally opposite corners of a ventilated cage (70 × 60 × 45 cm), with the foliage of the two plants ~50 cm apart. At 09.00 h, 20 female *T. chilonis* wasps were released in the center of the cage. After 48 h, wasps and whiteflies were removed. Parasitism was assessed after 7 d, and emerging offspring were counted 12 d after oviposition. In the second experiment, 30 newly hatched *P. rapae* larvae (0–24 h old) were introduced to each plant. After the caterpillars had settled, one group of infested plants was kept in a climate-controlled room, while another group was infested with 100 adult whiteflies, as described above. After 3 d, the number of caterpillars parasitized by 10 female *C. rubecula* wasps was assessed in a ventilated cage where wasps were given a choice between plants infested with caterpillars and those infested with both caterpillars and whiteflies. After 48 h, the

wasps and whiteflies were discarded, and parasitism was evaluated 7 d later by dissecting the larvae to check for developing parasitoids.

Statistical analysis

Binomial tests were performed to analyze the data from Y-tube olfactometer experiments. Fisher's protected least significant difference (PLSD) test of ANOVA was used to analyze the gene expression and phytohormone data. The number of parasitized eggs or caterpillars were statistically analyzed by one-way ANOVA. Changes in volatile emission were analyzed using projection to latent structures-discriminant analysis (PLS-DA) utilizing the software program SIMCA-P v10.5 (Umetrics AB, Umea, Sweden).

Results

Choices of parasitic wasps in the Y-tube olfactometer

We first examined the effects of *B. tabaci* density and infestation sequence by *P. rapae* caterpillars and *B. tabaci* on the attraction of *C. rubecula* wasps to caterpillar-induced plant volatiles. We also investigated the effects of *B. tabaci* density and infestation sequence by *P. rapae* eggs and *B. tabaci* on the preference of *T. chilonis* wasps for *P. rapae* egg-induced plant volatiles. At a density of 50 adults per plant, *B. tabaci* did not interfere with the attraction of *C. rubecula* wasps to caterpillar-infested plant volatiles, regardless of the feeding sequence (Fig. 1A). At a density of 75 adults per plant, *B. tabaci* feeding resulted in reduced *C. rubecula* attraction in the treatments of 'first caterpillars then whiteflies' as well as 'caterpillars and whiteflies simultaneously', but not in the treatment of 'first whiteflies then caterpillars' (Fig. 1A). However, at 100 adults per plant, *B. tabaci* interfered with *C. rubecula* attraction, irrespective of feeding sequence (Fig. 1A). In contrast, for none of the three densities tested or infestation sequences did *B. tabaci* affect the attraction of *T. chilonis* wasps to egg-infested plant volatiles (Fig. 1B). Based on these results, a density of 100 *B. tabaci* adults and simultaneous infestation with either *P. rapae* eggs or caterpillars was selected for subsequent bioassays.

Volatiles emitted by infested Arabidopsis plants

GC-MS analysis revealed that 30 volatile compounds were consistently released from all experimental plants infested, but with significant differences among treatments (Supplementary Table S2). PLS-DA yielded a model with two significant principal components (PCs; model statistics: R^2 $X = 0.75$, R^2 $Y = 0.46$, and $Q^2 = 0.58$), which largely separated the four treatments (Fig. 2). The first PC significantly separated treatments with caterpillars from those with caterpillars plus *B. tabaci* (Tukey's HSD multiple comparison tests, $P < 0.05$), whereas the second PC significantly separated treatments with caterpillars from those with eggs, regardless of *B. tabaci* presence (Tukey's HSD multiple comparison tests, $P < 0.05$; Fig. 2). Compared with undamaged plants, plants infested with eggs emitted higher amounts of methyl salicylate (MeSA) and (*E*)-β-caryophyllene (Supplementary Table S2).

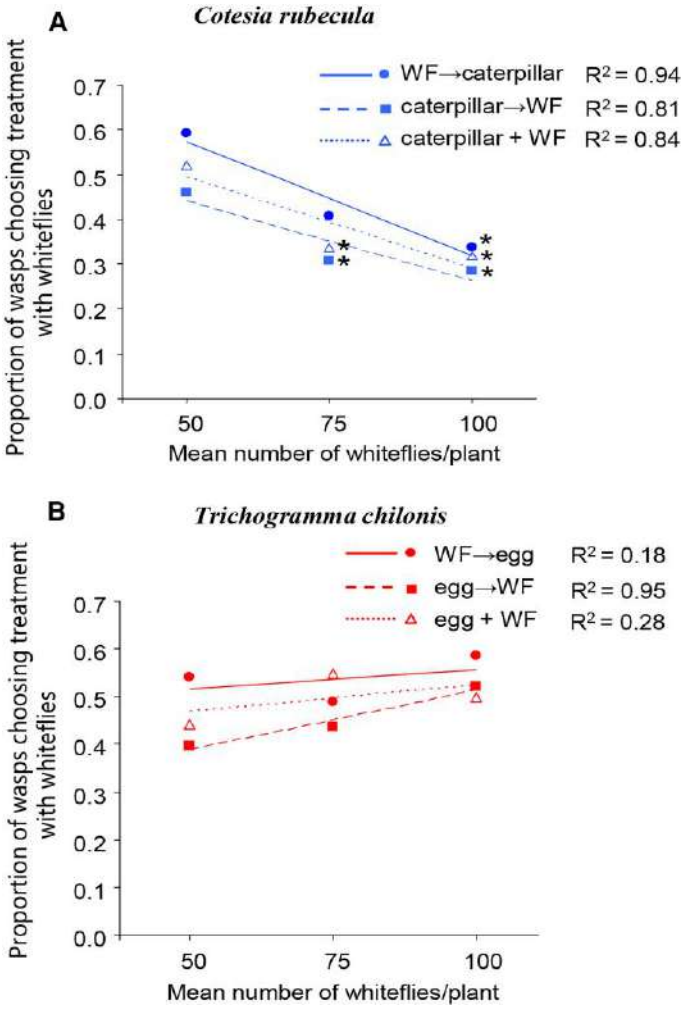

Fig. 1. Behavioral responses of larval or egg parasitoids in the Y-tube olfactometer. Attraction of the parasitoids to the volatiles emitted from plants infested with *P. rapae* caterpillars and *B. tabaci* (A) or *P. rapae* eggs and *B. tabaci* (B), when volatiles from plants infested by eggs or caterpillars alone were offered as an alternative. (A) Effect of whitefly density on the behavior of *C. rubecula* females for three different infestation sequences of *P. rapae* and *B. tabaci*. (B) Effect of whitefly density on the behavior of *T. chilonis* females for three different sequences of egg deposition and *B. tabaci* infestation. Wasp responses are presented as numbers of wasps that chose the treated plants (plus whitefly) divided by the total number of responding wasps ($n = 60$). Data points marked with an asterisk indicate significant differences from a 50:50 distribution (binomial test; *$P < 0.05$). WF, whitefly *B. tabaci*.

The volatile emissions from plants infested with eggs plus *B. tabaci* did not differ from those emitted by plants infested with eggs alone. In contrast, higher amounts of (*E*)-2-hexenal, (*Z*)-3-hexenyl acetate, MeSA, (*E*)-β-farnesene, and (*E*, *E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT) were emitted from caterpillar-infested plants compared with undamaged plants (Supplementary Table S2). However, plants infested by both caterpillars and *B. tabaci* emitted significantly lower amounts of (*E*)-2-hexenal, (*Z*)-3-hexenyl acetate,

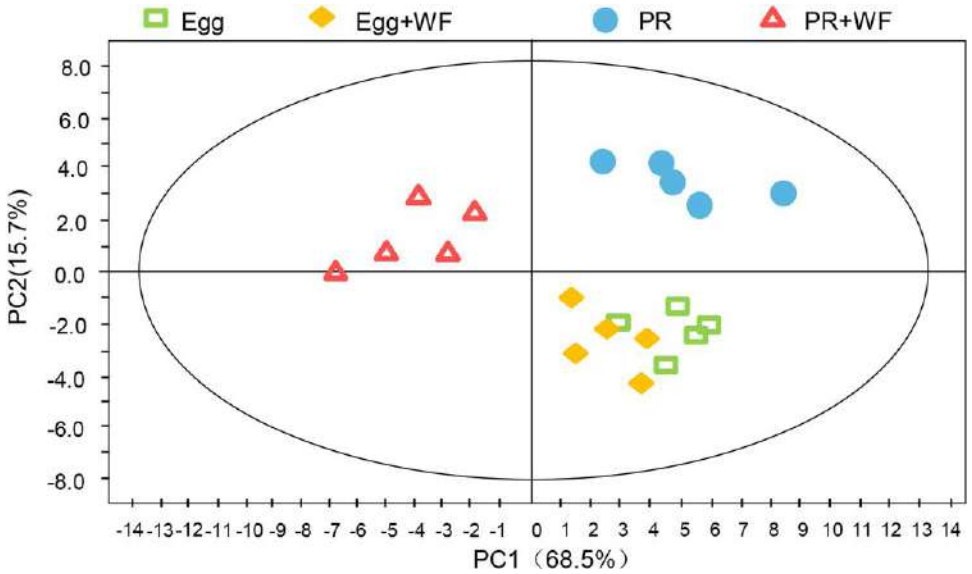

Fig. 2. Projection to latent structures-discriminant analysis (PLS-DA) of volatile emissions from Arabidopsis plants comparing different treatments. Score plot visualizing the grouping pattern of the samples according to the first two PLS components with the explained variance in parentheses. The ellipse defines Hotelling's T2 confidence region (95%). Egg, plant infested with *P. rapae* eggs for 3 d; Egg + WF, plant infested with *P. rapae* eggs and whiteflies for 3 d; PR, plant infested with *P. rapae* caterpillars for 3 d; PR + WF, plant infested with *P. rapae* caterpillars and whiteflies for 3 d.

(*E*)-β-farnesene, and TMTT compared with plants infested by caterpillars alone (Supplementary Table S2; Fig. 3A).

We further examined the effects of *B. tabaci* co-infestation on the caterpillar-induced emission of five volatile compounds from the mutants *NahG*, *npr1*, and *wrky70*. Neither caterpillar infestation nor dual infestation induced the emission of MeSA from *NahG* plants, and the amounts of (*E*)-2-hexenal, (*Z*)-3-hexenyl acetate, (*E*)-β-farnesene, or TMTT emitted from dual-infested *NahG* plants were not significantly different from those emitted by *NahG* plants infested only with caterpillars (Fig. 3B). Similarly, the emissions from *npr1* plants did not differ for the five volatile compounds when comparing dual-infested plants with plants that were only infested with caterpillars (Fig. 3C). In contrast, the amounts of (*E*)-2-hexenal, (*Z*)-3-hexenyl acetate, and TMTT emitted from dual-infested *wrky70* plants were significantly lower than those from *wrky70* plants with only caterpillars (ANOVA; $P = 0.028$, 0.004, and 0.052, respectively; Fig. 3D).

Quantification of endogenous jasmonic acid and salicylic acid

At 12 h, but not 72 h, after infestation, the amount of JA was significantly lower in leaves infested with both caterpillars and *B. tabaci* compared with leaves infested with caterpillars alone (ANOVA, $P = 0.025$; Fig. 4A). In contrast, at 72 h post-infestation, but not at 12 h, the amount of SA was significantly higher in leaves co-infested with caterpillars and *B. tabaci* compared with those infested with caterpillars alone (ANOVA, $P = 0.041$; Fig. 4B).

In leaves infested with *P. rapae* eggs and *B. tabaci* simultaneously, the amount of JA at 12 h or 72 h did not differ from the levels in leaves infested with *P. rapae* eggs alone (Fig. 5A). Similarly, the amount of SA in leaves co-infested with *P. rapae* eggs and *B. tabaci* at 12 h or 72 h was not significantly different from that in leaves infested with *P. rapae* eggs alone (Fig. 5B).

Gene expression changes in response to infestations with *P. rapae* eggs, caterpillars, or *B. tabaci*

We quantified the transcript levels of six genes in leaves co-infested with *B. tabaci* and *P. rapae* eggs or caterpillars, compared with leaves infested with *P. rapae* eggs or caterpillars alone. *Phytoalexin deficient4* (*PAD4*) and *Enhanced disease susceptibility1* (*EDS1*) are two genes known to function upstream of SA signaling (Zarate *et al.*, 2007). *Lipoxygenase2* (*LOX2*) is a key enzyme in the biosynthesis of JA via the octadecanoid pathway (Bell *et al.*, 1995), while *12-Oxophytodienoate reductase 3* (*OPR3*) is an isoenzyme involved in JA biosynthesis (Schaller *et al.*, 2000). Additionally, *NPR1* and *WRKY70* are two important transcription factor genes that modulate SA/JA crosstalk in Arabidopsis (Spoel *et al.*, 2003; Li *et al.*, 2004). *Pieris rapae* caterpillar infestation was found to cause a significant induction of *LOX2* and *OPR3* transcript levels (ANOVA: *LOX2*, $P = 0.007$; *OPR3*, $P = 0.025$), but significantly reduced *WRKY70* transcript levels (ANOVA; *WRKY70*, $P = 0.035$; Fig. 4C). Co-infestation with *B. tabaci* resulted in a significant induction of *EDS1*, *PAD4*, *WRKY70*, and *NPR1* transcript levels (ANOVA: *EDS1*, $P = 0.019$; *PAD4*,

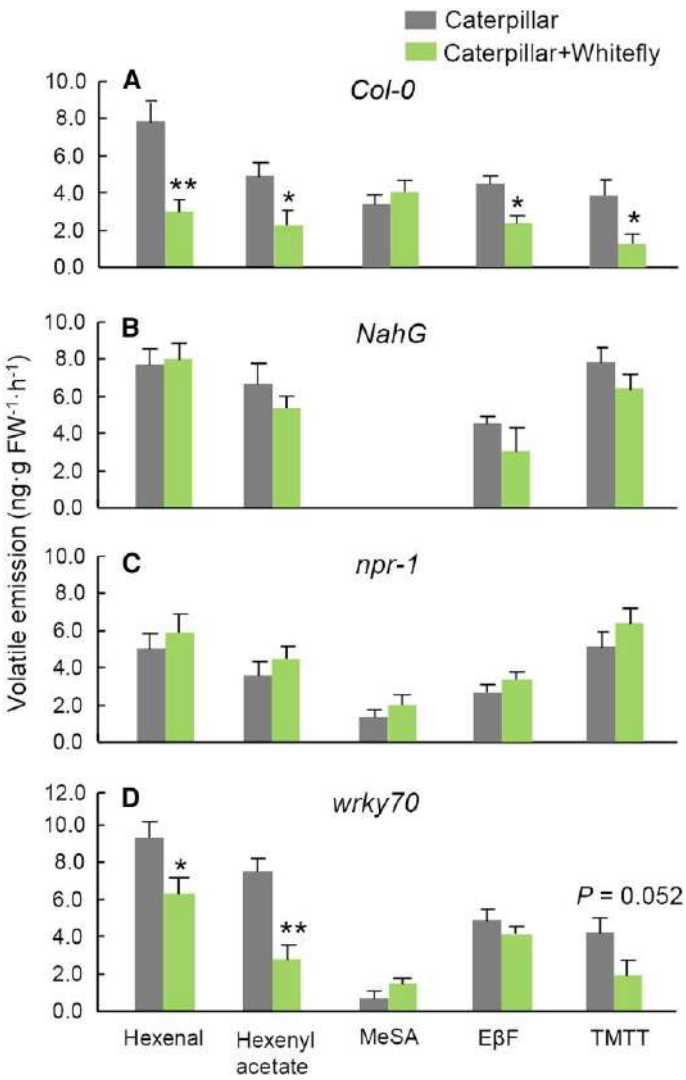

Fig. 3. Effect of whitefly co-infestation on the emission of volatile compounds induced by *P. rapae* caterpillars. Comparison of mean (±SE) ($n = 5$) emission rates of the five main volatile compounds from plants infested with *P. rapae* caterpillars only and plants simultaneously infested with *P. rapae* caterpillars and *B. tabaci*. Values are means ± SE ($n = 5$). Asterisks represent significant differences (*$P < 0.05$; **$P < 0.01$) from caterpillar-infested plants as determined by Fisher protected least significant difference (PLSD) test of ANOVA. Hexenal, (*E*)-2-hexenal; hexenyl acetate, (*Z*)-3-hexenyl acetate; MeSA, methyl salicylate; EβF, (*E*)-β-farnesene; TMTT, (*E*, *E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene.

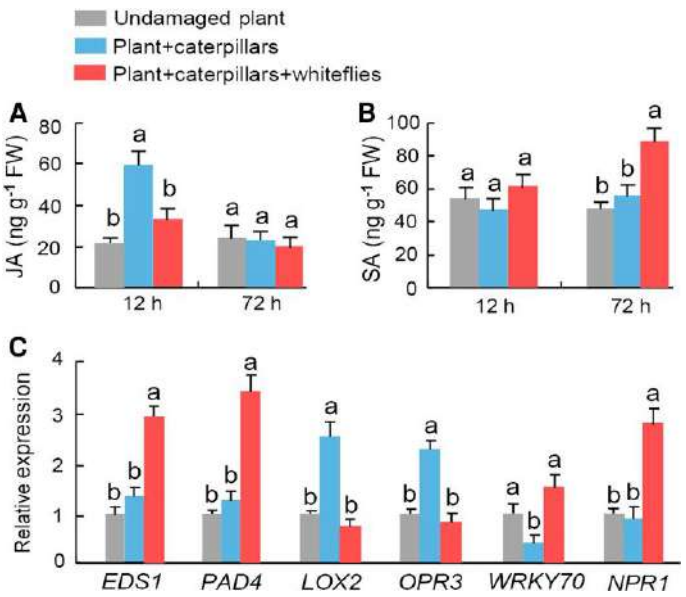

Fig. 4. Effects of whitefly infestation on caterpillar-induced plant defensive responses. (A) JA levels in leaves from undamaged plants, plants infested with *P. rapae* larvae only, and those infested with *P. rapae* larvae and *B. tabaci* adults simultaneously. (B) SA levels in leaves from undamaged plants, plants infested with *P. rapae* larvae only, and those infested with *P. rapae* larvae and *B. tabaci* adults simultaneously. (C) Expression levels of JA- and SA-regulated genes, and two transcription factors involved in mediating JA-SA crosstalk in leaves from undamaged plants, plants infested with *P. rapae* larvae only, and those infested with *P. rapae* larvae and *B. tabaci* adults simultaneously. Gene expression was measured 72 h after infestation. Values are means ± SE ($n = 4$). Different letters above bars indicate a significant difference in transcript levels between treatments (Fisher's PLSD test of ANOVA, $P < 0.05$).

$P < 0.001$; *WRKY70*, $P = 0.028$; *NPR1*, $P = 0.019$), while significantly reducing the *LOX2* and *OPR3* transcript levels induced by *P. rapae* caterpillars (ANOVA: *LOX2*, $P < 0.001$; *OPR3*, $P = 0.011$; Fig. 4C).

Pieris rapae egg infestation caused a significant induction of *EDS1*, *PAD4*, and *NPR1* transcript levels (ANOVA: *EDS1*, $P = 0.032$; *PAD4*, $P = 0.009$; *NPR1*, $P = 0.032$), but significantly reduced *WRKY70* transcript levels (ANOVA: *WRKY70*, $P = 0.023$; Fig. 5C). Co-infestation with *B. tabaci* did not affect the transcript levels of these six genes induced by *P. rapae* eggs (Fig. 5C), in clear contrast to the results for the genes induced by caterpillar infestation.

Effects of salicylic acid signaling and associated transcription factors on the attraction of the larval parasitoid

To investigate whether SA signaling, or the transcription factors NPR1 or WRKY70, are involved in the effects of *B. tabaci* co-infestation on the attraction of the larval parasitoid *C. rubecula*, we compared the attractiveness of Col-0 plants and the three mutants: *NahG* (SA-deficient; Delaney *et al.*, 1994), *npr1*, and *wrky70*. In a two-choice olfactometer assay, female *C. rubecula* wasps preferred the volatiles from Col-0 plants infested by caterpillars alone over those from Col-0 plants co-infested with caterpillars and *B. tabaci* (binomial test, $P = 0.007$; Fig. 6). A similar preference was observed with volatiles from *wrky70* plants infested by caterpillars (binomial test, $P = 0.036$; Fig. 6). However, *C. rubecula* did not discriminate between volatiles from caterpillar-infested *NahG* plants and dual-infested *NahG* plants, nor did they show a preference when given a similar choice with *npr1* plants (Fig. 6). These

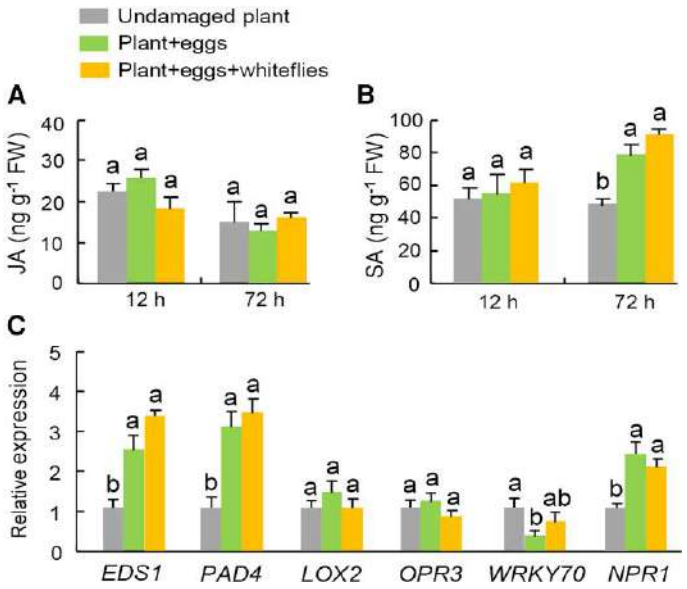

Fig. 5. Effects of whitefly infestation on egg-induced plant defensive responses. (A) JA levels in leaves from undamaged plants, plants infested with *P. rapae* eggs only, and those infested with *P. rapae* eggs and *B. tabaci* adults simultaneously. (B) SA levels in leaves from undamaged plants, plants infested with *P. rapae* eggs only, and those infested with *P. rapae* eggs and *B. tabaci* adults simultaneously. (C) Expression levels of JA- and SA-regulated genes, and two transcription factors involved in mediating JA-SA crosstalk in leaves from undamaged plants, plants infested with *P. rapae* eggs only, and those infested with *P. rapae* eggs and *B. tabaci* adults simultaneously. Gene expression was measured 72 h after infestation. Values are means ± SE ($n = 4$). Different letters above bars indicate significant difference in transcript levels between treatments (Fisher's PLSD test of ANOVA, $P < 0.05$).

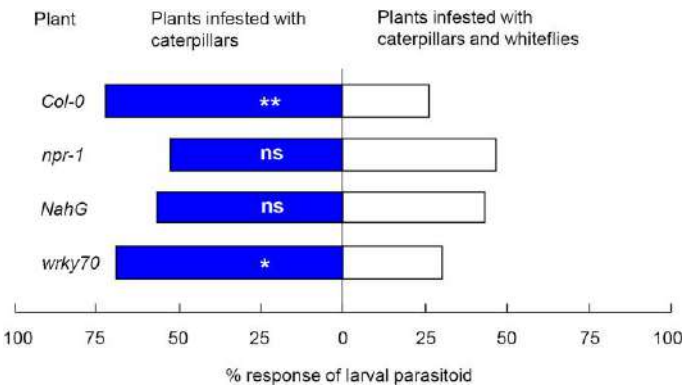

Fig. 6. Behavioral responses of female *Cotesia rubecula* wasps choosing between volatiles emitted from plants infested with *P. rapae* caterpillars only and plants simultaneously infested with *P. rapae* caterpillars and *B. tabaci* in a Y-tube olfactometer. Bars represent the percentages of wasps choosing either of the odor sources. Choices between odor sources were statistically analyzed with a two-sided binomial test (**$P < 0.01$; ns, not significant).

results demonstrate that interference by *B. tabaci* with caterpillar-induced indirect defenses is mediated by SA in an NPR1-dependent manner.

Recruitment of parasitoids under greenhouse conditions

The number of parasitized *P. rapae* eggs on plants co-infested with eggs and *B. tabaci* did not differ from the number recorded on plants infested with eggs alone (Fig. 7A). In contrast, the number of parasitized *P. rapae* caterpillars on plants co-infested with caterpillars and *B. tabaci* was significantly lower than on plants infested with caterpillars alone (Fig. 7B). These data confirm that *B. tabaci* co-infestation interferes with the recruitment of the larval parasitoid *C. rubecula*, but not of the egg parasitoid *T. chilonis*.

Discussion

In nature, plants usually face simultaneous or sequential attacks by multiple herbivores. Depending on the feeding guild of the herbivores, different signaling pathways are activated, leading to specific defense responses. Crosstalk between signaling pathways may provide plants with a sophisticated mechanism to fine-tune their defenses against various attackers (Bostock, 2005; Pieterse *et al.*, 2012). For example, SA/JA crosstalk can be either mutually antagonistic or synergistic, resulting in negative or positive functional outcomes (Pieterse and Dicke, 2007). Research on the effects of multiple herbivory has focused on direct plant defenses and interspecific competition within ecological communities (Kaplan and Denno, 2007; Soler *et al.*, 2012; Liu *et al.*, 2021), while the effects on plant volatile emissions and the attraction of natural enemies have received little attention (Dicke *et al.*, 2009).

In this study we found that whitefly co-infestation interfered with the attraction of the larval parasitoid *C. rubecula* to volatiles emitted by Arabidopsis plants infested with *P. rapae* caterpillars, regardless of the feeding sequence (Fig. 1A). This is consistent with previous findings that *B. tabaci* infestation disrupts indirect defenses induced by *Plutella xylostella*, which activates JA signaling in Arabidopsis plants (Zhang *et al.*, 2013b). We further demonstrated that whitefly co-infestation strongly affects the composition of plant volatiles induced by *P. rapae* caterpillars (Fig. 2). Among the four volatile compounds suppressed by *B. tabaci* co-infestation (Supplementary Table S2), (*E*)-2-hexenal and (*Z*)-3-hexenyl acetate are known to be regulated by JA signaling (Joo *et al.*, 2018) and are attractive to *Cotesia* wasps (Shiojiri *et al.*, 2006). Additionally, we provide evidence that *B. tabaci* infestation not only suppressed the expression of two key genes, *LOX2* and *OPR3*, involved in JA biosynthesis (Bell *et al.*, 1995; Schaller *et al.*, 2000), but also reduced JA accumulation triggered by *P. rapae* caterpillars (Fig. 4A, C). Combined, these results show that the interference of *B. tabaci* with *P. rapae*-induced indirect plant defenses is due to the suppression of the JA signaling pathway, which in turn reduces the emission of JA-regulated volatiles. It should be noted that placing small insects in clip cages can reduce their fitness and feeding rate (Martinez-Chavez *et al.*, 2024). This was probably also the case for *B. tabaci* in our experiments.

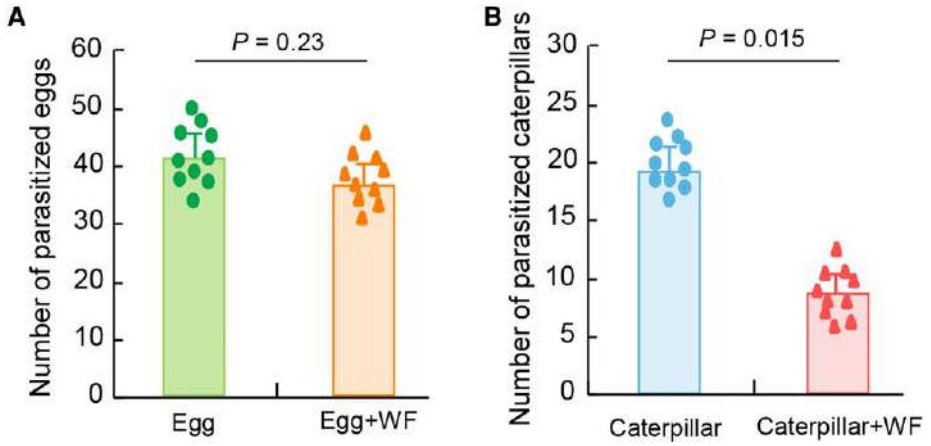

Fig. 7. Effect of whitefly infestation on the parasitism rate of egg or larval parasitoids in the greenhouse. (A) Mean number (±SE, $n = 10$) of parasitized eggs in the two-choice bioassay when *T. chilonis* females were provided with plants infested by *P. rapae* eggs only and plants simultaneously infested by *P. rapae* eggs and *B. tabaci*. (B) Mean number (±SE, $n = 10$) of parasitized caterpillars in the two-choice bioassay when *C. rubecula* females were provided with the plants infested by *P. rapae* caterpillars only and plants simultaneously infested by *P. rapae* caterpillars and *B. tabaci*. The numbers of parasitized eggs or caterpillars in different treatments were analyzed by one-way ANOVA. WF, whitefly.

Considering that the whiteflies probably would feed more under less confined conditions, the observed impact on the volatile emissions and parasitoid attraction can be expected to be stronger in nature than what we observed.

The induction of plant volatiles by chewing insects is primarily regulated by the JA signaling pathway (Thaler, 1999; Ament *et al.*, 2004). Therefore, the inhibition of JA synthesis or its action will reduce volatile emissions and interfere with the attraction of parasitoids to caterpillar-damaged plants (van Poecke and Dicke, 2002; Kessler *et al.*, 2004; Wei *et al.*, 2011). Studies on phloem-feeding insects, which induce the SA pathway, have found them to suppress the emission of JA-regulated volatiles and disrupt the attraction of parasitoids or predators targeting herbivores that activate the JA pathway. For instance, cotton plants co-infested by *Spodoptera exigua* caterpillars, which induce the JA pathway (Al-Zahrani *et al.*, 2020), and the whitefly *B. tabaci* emit lower amounts of volatiles compared with plants infested solely by caterpillars (Rodriguez-Saona *et al.*, 2003). Similarly, by suppressing the emission of the JA-regulated monoterpene (*E*)-β-ocimene (Arimura *et al.*, 2008), *B. tabaci* co-infestation leads to reduced attraction of predatory mites to Lima bean plants simultaneously infested with the spider mite *Tetranychus urticae* and *B. tabaci* (Zhang *et al.*, 2009). However, the mechanisms underlying the interference of multiple herbivory with plant volatile emissions and indirect plant defenses remain unclear, although SA and ET signaling have been suggested to play roles (Zhang *et al.*, 2009, 2013b). We show that *B. tabaci*-induced suppression of JA-dependent responses (Fig. 4A, C) is associated with the induction of SA-dependent responses, including SA accumulation and up-regulation of two SA-dependent defense genes, *EDS1* and *PAD4* (Fig. 4B, C). Using SA-deficient *NahG* mutant plants, we confirmed that SA accumulation is required for the reduced emission of the JA-regulated volatiles (*E*)-2-hexenal and (*Z*)-3-hexenyl acetate (Fig. 3), as well as for the interference with the attraction of a parasitoid of *P. rapae* caterpillars (Fig. 6). These findings lead us to conclude that *B. tabaci* interferes with JA-regulated indirect defenses against *P. rapae* caterpillars via antagonistic crosstalk between the JA and SA signaling pathways.

NPR1 and WRKY70 are two key transcription factors involved in the crosstalk between JA and SA signaling pathways (Spoel *et al.*, 2003; Li *et al.*, 2004). While NPR1 is active in the nucleus, it also functions in the cytosol, where it negatively interacts with several components of the JA pathway (Spoel *et al.*, 2003; Pieterse and Van Loon, 2004). WRKY70 acts downstream of NPR1 and is crucial for the SA-mediated suppression of JA-responsive gene expression (Li *et al.*, 2004). In the present study, we found an increased expression of NPR1 and WRKY70 in plants simultaneously infested with caterpillars and *B. tabaci* (Fig. 4C), suggesting that one or both transcription factors may be involved in the interference by *B. tabaci* with caterpillar-induced volatile emissions. Indeed, we found that *B. tabaci* did not interfere with parasitoid attraction in *npr1* mutant plants (Fig. 6), which was consistent with the absence of a reduction in the emission of key volatiles induced by caterpillars (Fig. 3C). Conversely, in *wrky70* mutant plants, *B. tabaci* still suppressed the emission of (*E*)-2-hexenal and (*Z*)-3-hexenyl acetate (Fig. 3D) and reduced the attractiveness of the plants to parasitoids (Fig. 6). Combined, these results imply that interference by *B. tabaci* with caterpillar-induced indirect defenses is dependent on NPR1 but not WRKY70. This finding is in agreement with the findings of a previous study in which the *npr1* mutation did not affect the expression of *WRKY70* in Arabidopsis (Li *et al.*, 2004).

Egg deposition by herbivorous insects has been shown to induce the emission of leaf volatiles that attract egg parasitoids (Hilker and Meiners, 2006; Fatouros *et al.*, 2014). We show

here that *P. rapae* egg deposition also induces the emission of higher amounts of MeSA and (*E*)-β-caryophyllene (Supplementary Table S2) and that the volatile blend emitted by egg-infested plants is attractive to the egg parasitoid *T. chilonis* (Supplementary Fig. S1A). We further demonstrate that *P. rapae* egg deposition induces SA accumulation and enhances the expression of two SA-dependent genes, *EDS1* and *PAD4* (Fig. 5B, C). This is consistent with previous findings that *P. brassicae* eggs activate the expression of the SA-marker gene *PR-1* in Arabidopsis (Little *et al.*, 2007; Lortzing *et al.*, 2020) and induce SA accumulation at the site of oviposition (Bruessow *et al.*, 2010; Valsamakis *et al.*, 2020). Given that *B. tabaci* induces SA-dependent responses both locally and systemically in Arabidopsis (Van de Ven *et al.*, 2000; Zhang *et al.*, 2013a), it was expected that *B. tabaci* co-infestation would have an additive effect on the volatile emission induced by *P. rapae* eggs. This turned out not to be the case as *B. tabaci* co-infestation did not affect the composition or quantity of volatile blends induced by *P. rapae* eggs (Supplementary Table S2; Fig. 2) and did not enhance the attraction of *T. chilonis* to egg-infested plants (Fig. 1B).

For our greenhouse trials, we switched from *A. thaliana*, used in the laboratory bioassays, to the more relevant cultivated plant *B. oleracea*. For both plant species, it has been shown that JA-dependent genes (e.g. *LOX2* and *AOS*) are up-regulated in parallel following *P. rapae* caterpillar attack (Reymond *et al.*, 2004; Broekgaarden *et al.*, 2007). The greenhouse results showed that *B. tabaci* also similarly affected the attractiveness of *B. oleracea* to the two parasitoids (Fig. 7). This finding holds significant relevance for various agroecosystems, as field surveys have shown that *B. tabaci* and *P. rapae* often co-occur on cabbage plants (Zhang *et al.*, 2013). Given the wide distribution of *B. tabaci* and its often much higher field density than used in the present study (Liu, 2000), it is likely that under natural conditions the interference by *B. tabaci* with plant defenses against other herbivores could be more pronounced than previously anticipated. Therefore, studies like ours seem important if we wish to gain detailed knowledge about the consequences of multiple herbivores attacking the same plants in a tri-trophic context. Such insights can be helpful when developing integrated pest management (IPM) strategies that aim to maximize the efficacy of parasitoids while minimizing the negative impacts of multiple herbivore species. To refine pest control approaches, further investigations into the interactions between simultaneously or sequentially attacking herbivores and their associated parasitoids under realistic field conditions are warranted. Such research could provide crucial guidance for the effective deployment of parasitoids in IPM programs (Dicke *et al.*, 2009).

Supplementary data

The following supplementary data are available at *JXB* online.

Fig. S1. Response of egg or larval parasitoids to the volatiles of plants from different treatments in a Y-tube olfactometer.

Table S1. Specific primers used for gene expression by qPCR.

Table S2. Concentration (ng g FW^{-1} h^{-1}) of volatile compounds detected in the headspace of Arabidopsis plants from different treatments.

Author contributions

PJZ, DWX, and YHL: conceptualization; YMD, YLS, and SZW: investigation and analysis; PJZ and TCJT: writing.

Conflict of interest

The authors declare no conflict of interest.

Funding

This work was financially supported by the National Natural Science Foundation of China (32172402 and 32402386), and the Science and Technology Innovation Project for Overseas Students in Nanjing city (2024) (009053501230348).

Data availability

All data supporting the findings of this study are available within the paper and within its supplementary data published online.

References

Al-Zahrani W, Bafeel SO, El-Zohri M. 2020. Jasmonates mediate plant defense responses to *Spodoptera exigua* herbivory in tomato and maize foliage. Plant Signaling & Behavior **15**, 1746898.

Ament K, Kant MR, Sabelis MW, Haring MA, Schuurink RC. 2004. Jasmonic acid is a key regulator of spider mite-induced volatile terpenoid and methyl salicylate emission in tomato. Plant Physiology **135**, 2025-2037.

Arimura G, Köpke S, Kunert M, Volpe V, David A, Brand P, Dabrowska P, Maffei ME, Boland W. 2008. Effects of feeding *Spodoptera littoralis* on Lima bean leaves: IV. Diurnal and nocturnal damage differentially initiate plant volatile emission. Plant Physiology **146**, 965-973.

Bell E, Creelman RA, Mullet JE. 1995. A chloroplast lipoxygenase is required for wound-induced jasmonic acid accumulation in Arabidopsis. Proceedings of the National Academy of Sciences, USA **92**, 8675-8679.

Bezemer TM, van Dam NM. 2005. Linking aboveground and belowground interactions via induced plant defenses. Trends in Ecology & Evolution **20**, 617-624.

Bostock RM. 2005. Signal crosstalk and induced resistance: straddling the line between cost and benefit. Annual Review of Phytopathology **43**, 545-580.

Broekgaarden C, Poelman EH, Steenhuis G, Voorrips RE, Dicke M, Vosman B. 2007. Genotypic variation in genome-wide transcription profiles induced by insect feeding: *Brassica oleracea–Pieris rapae* interactions. BMC Genomics **8**, 239.

Bruessow F, Gouhier-Darimont C, Buchala A, Metraux JP, Reymond P. 2010. Insect eggs suppress plant defence against chewing herbivores. The Plant Journal **62**, 876-885.

Chen CS, Zhao C, Wu ZY, Liu GF, Yu XP, Zhang PJ. 2021. Whitefly-induced tomato volatiles mediate host habitat location of the parasitic wasp *Encarsia formosa*, and enhance its efficacy as a bio-control agent. Pest Management Science **77**, 749-757.

Clavijo McCormick A, Unsicker SB, Gershenzon J. 2012. The specificity of herbivore-induced plant volatiles in attracting herbivore enemies. Trends in Plant Science **17**, 303-310.

Cusumano A, Weldegergis BT, Colazza S, Dicke M, Fatouros NE. 2015. Attraction of egg-killing parasitoids toward induced plant volatiles in a multi-herbivore context. Oecologia **179**, 163-174.

Czechowski T, Stitt M, Altmann T, Udvardi MK, Scheible WR. 2005. Genome-wide identification and testing of superior reference genes for transcript normalization in Arabidopsis. Plant Physiology **139**, 5-17.

Delaney TP, Uknes S, Vernooij B, *et al.* 1994. A central role of salicylic acid in plant disease resistance. Science **266**, 1247-1250.

Dicke M, Sabelis MW. 1988. How plants obtain predatory mites as bodyguards. Netherlands Journal of Zoology **38**, 148-165.

Dicke M, van Loon JJ, Soler R. 2009. Chemical complexity of volatiles from plants induced by multiple attack. Nature Chemical Biology **5**, 317-324.

Dicke M, Baldwin IT. 2010. The evolutionary context for herbivore-induced plant volatiles: beyond the 'cry for help'. Trends in Plant Science **15**, 167-175.

De Vos M, Van Zaanen W, Koornneef A, Korzelius JP, Dicke M, Van Loon LC, Pieterse CM. 2006. Herbivore-induced resistance against microbial pathogens in *Arabidopsis*. Plant Physiology **142**, 352-363.

Erb M, Reymond P. 2019. Molecular interactions between plants and insect herbivores. Annual Review of Plant Biology **70**, 527-557.

Fatouros NE, Pineda A, Huigens ME, Broekgaarden C, Shimwela MM, Figueroa Candia IA, Verbaarschot P, Bukovinszky T. 2014. Synergistic effects of direct and indirect defences on herbivore egg survival in a wild crucifer. Proceedings of the Royal Society B: Biological Sciences **281**, 20141254.

Hilker M, Meiners T. 2006. Early herbivore alert: insect eggs induce plant defense. Journal Chemical Ecology **32**, 1379-1397.

Hilker M, Fatouros NE. 2015. Plant responses to insect egg deposition. Annual Review of Entomology **60**, 493-515.

Howe GA, Jander G. 2008. Plant immunity to insect herbivores. Annual Review of Plant Biology **59**, 41-66.

Hu X, Su S, Liu Q, Jiao Y, Peng Y, Li Y, Turlings TC. 2020. Caterpillar-induced rice volatiles provide enemy-free space for the offspring of the brown planthopper. eLife **9**, e55421.

Joo Y, Schuman MC, Goldberg JK, Wissgott A, Kim SG, Baldwin IT. 2018. Herbivory elicits changes in green leaf volatile production via jasmonate signaling and the circadian clock. Plant, Cell & Environment **42**, 972-982.

Kaplan I, Denno RF. 2007. Interspecific interactions in phytophagous insects revisited: a quantitative assessment of competition theory. Ecology Letters **10**, 977-994.

Kempema LA, Cui X, Holzer FM, Walling LL. 2007. Arabidopsis transcriptome changes in response to phloem-feeding silverleaf whitefly nymphs. Similarities and distinctions in responses to aphids. Plant Physiology **143**, 849-865.

Kessler A, Baldwin IT. 2001. Defensive function of herbivore-induced plant volatile emissions in nature. Science **291**, 2141-2144.

Kessler A, Baldwin IT. 2002. Plant responses to insect herbivory: the emerging molecular analysis. Annual Review of Plant Biology **53**, 299-328.

Kessler A, Halitschke R, Baldwin IT. 2004. Silencing the jasmonate cascade: induced plant defenses and insect populations. Science **305**, 665-668.

Koornneef A, Pieterse CMJ. 2008. Cross talk in defense signaling. Plant Physiology **146**, 839-844.

Li J, Brader G, Palva ET. 2004. The WRKY70 transcription factor: a node of convergence for jasmonate-mediated and salicylate-mediated signals in plant defense. The Plant Cell **16**, 319-331.

Ling X, Gu S, Tian C, Guo H, Degen T, Turlings TCJ, Ge F, Sun Y. 2021. Differential levels of fatty acid-amino acid conjugates in the oral secretions of lepidopteran larvae account for the different profiles of volatiles. Pest Management Science **77**, 3970-3979.

Little D, Gouhier-Darimont C, Bruessow F, Reymond P. 2007. Oviposition by pierid butterflies triggers defense responses in *Arabidopsis*. Plant Physiology **143**, 784-800.

Liu Q, Hu X, Su S, Ning Y, Peng Y, Ye G, Lou Y, Turlings TCJ, Li Y. 2021. Cooperative herbivory between two important pests of rice. Nature Communications **12**, 6772.

Liu TX. 2000. Population dynamics of *Bemisia argentifolii* (Homoptera: Aleyrodidae) on spring collard and relationship to yield in the lower Rio Grande valley of Texas. Journal of Economic Entomology **93**, 750-756.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods **25**, 402-408.

Lortzing T, Kunze R, Steppuhn A, Hilker M, Lortzing V. 2020. Arabidopsis, tobacco, nightshade and elm take insect eggs as herbivore alarm and show similar transcriptomic alarm responses. Scientific Reports **10**, 16281.

Martinez-Chavez LM, Roberts JM, Karley AJ, Shaw B, Pope TW. 2024. The clip cage conundrum: assessing the interplay of confinement method and aphid genotype in fitness studies. Insect Science **31**, 1591-1602.

Matsui K. 2006. Green leaf volatiles: hydroperoxide lyase pathway of oxylipin metabolism. Current Opinion in Plant Biology **9**, 274-280.

Moayeri HRS, Ashouri A, Poll L, Enkegaard A. 2007. Olfactory response of a predatory mirid to herbivore induced plant volatiles: multiple herbivory vs. single herbivory. Journal of Applied Entomology **131**, 326-332.

Pashalidou FG, Huigens ME, Dicke M, Fatouros NE. 2010. The use of oviposition-induced plant cues by *Trichogramma* egg parasitoids. Ecological Entomology **35**, 748-753.

Pieterse CM, Van Loon LC. 2004. NPR1: the spider in the web of induced resistance signaling pathways. Current Opinion in Plant Biology **7**, 456-464.

Pieterse CM, Dicke M. 2007. Plant interactions with microbes and insects: from molecular mechanisms to ecology. Trends in Plant Science **12**, 564-569.

Pieterse CM, Van der Does D, Zamioudis C, Leon-Reyes A, Van Wees SC. 2012. Hormonal modulation of plant immunity. Annual Review of Cell and Developmental Biology **28**, 489-521.

Ponzio C, Cascone P, Cusumano A, Weldegergis BT, Fatouros NE, Guerrieri E, Dicke M, Gols R. 2016. Volatile-mediated foraging behaviour of three parasitoid species under conditions of dual insect herbivore attack. Animal Behaviour **111**, 197-206.

Rasmann S, Turlings TC. 2007. Simultaneous feeding by aboveground and belowground herbivores attenuates plant-mediated attraction of their respective natural enemies. Ecology Letters **10**, 926-936.

Reymond P, Weber H, Damond M, Farmer EE. 2000. Differential gene expression in response to mechanical wounding and insect feeding in *Arabidopsis*. The Plant Cell **12**, 707-720.

Reymond P, Bodenhausen N, Van Poecke RM, Krishnamurthy V, Dicke M, Farmer EE. 2004. A conserved transcript pattern in response to a specialist and a generalist herbivore. The Plant Cell **16**, 3132-3147.

Rodriguez-Saona C, Crafts-Brandner SJ, Cañas LA. 2003. Volatile emissions triggered by multiple herbivore damage: beet armyworm and whitefly feeding on cotton plants. Journal of Chemical Ecology **29**, 2539-2550.

Rodriguez-Saona C, Chalmers JA, Raj S, Thaler JS. 2005. Induced plant responses to multiple damagers: differential effects on an herbivore and its parasitoid. Oecologia **143**, 566-577.

Ruther J, Kleier S. 2005. Plant-plant signaling: ethylene synergizes volatile emission in *Zea mays* induced by exposure to (*Z*)-3-hexen-1-ol. Journal of Chemical Ecology **31**, 2217-2222.

Schaller F, Biesgen C, Müssig C, Altmann T, Weiler EW. 2000. 12-Oxophytodienoate reductase 3 (*OPR3*) is the isoenzyme involved in jasmonate biosynthesis. Planta **210**, 979-984.

Schmelz EA. 2015. Impacts of insect oral secretions on defoliation-induced plant defense. Current Opinion in Insect Science **9**, 7-15.

Shiojiri K, Kishimoto K, Ozawa R, Kugimiya S, Urashimo S, Arimura G, Horiuchi J, Nishioka T, Matsui K, Takabayashi J. 2006. Changing green leaf volatile biosynthesis in plants: an approach for improving plant resistance against both herbivores and pathogens. Proceedings of the National Academy of Sciences, USA **103**, 16672-16676.

Soler R, Badenes-Pérez FR, Broekgaarden C, Zheng SJ, David A, Boland W, Dicke M. 2012. Plant-mediated facilitation between a leaf-feeding and a phloem-feeding insect in a brassicaceous plant: from insect performance to gene transcription. Functional Ecology **26**, 156-166.

Spoel SH, Koornneef A, Claessens SM, *et al.* 2003. NPR1 modulates cross-talk between salicylate- and jasmonate-dependent defense pathways through a novel function in the cytosol. The Plant Cell **15**, 760-770.

Spoel SH, Johnson JS, Dong X. 2007. Regulation of tradeoffs between plant defenses against pathogens with different lifestyles. Proceedings of the National Academy of Sciences, USA **104**, 18842-18847.

Steinberg S, Dicke M, Vet LE. 1993. Relative importance of infochemicals from 1st and 2nd trophic level in long-range host location by the larval parasitoid *Cotesia glomerata*. Journal of Chemical Ecology **19**, 47-59.

Thaler JS. 1999. Jasmonate-inducible plant defences cause increased parasitism of herbivores. Nature **399**, 686-687.

Thaler JS, Humphrey PT, Whiteman NK. 2012. Evolution of jasmonate and salycilate signal crosstalk. Trends in Plant Science **17**, 260-270.

Turlings TCJ, Tumlinson JH, Lewis WJ. 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. Science **250**, 1251-1253.

Turlings TCJ, Erb M. 2018. Tritrophic interactions mediated by herbivore-induced plant volatiles: mechanisms, ecological relevance, and application potential. Annual Review of Entomology **63**, 433-452.

Valsamakis G, Bittner N, Fatouros NE, Kunze R, Hilker M, Lortzing V. 2020. Priming by timing: *Arabidopsis thaliana* adjusts its priming response to lepidoptera eggs to the time of larval hatching. Frontiers in Plant Science **11**, 619589.

Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F. 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biology **3**, RESEARCH0034.

Van de Ven WT, LeVesque CS, Perring TM, Walling LL. 2000. Local and systemic changes in squash gene expression in response to silverleaf whitefly feeding. The Plant Cell **12**, 1409-1423.

van Poecke RM, Dicke M. 2002. Induced parasitoid attraction by *Arabidopsis thaliana*: involvement of the octadecanoid and the salicylic acid pathway. Journal of Experimental Botany **53**, 1793-1799.

van Wees SC, De Swart EA, Van Pelt JA, Van Loon LC, Pieterse CM. 2000. Enhancement of induced disease resistance by simultaneous activation of salicylate- and jasmonate-dependent defense pathways in *Arabidopsis thaliana*. Proceedings of the National Academy of Sciences, USA **97**, 8711-8716.

Wei J, Wang L, Zhao J, Li C, Ge F, Kang L. 2011. Ecological trade-offs between jasmonic acid-dependent direct and indirect plant defences in tritrophic interactions. New Phytologist **189**, 557-567.

Zarate SI, Kempema LA, Walling LL. 2007. Silverleaf whitefly induces salicylic acid defenses and suppresses effectual jasmonic acid defenses. Plant Physiology **143**, 866-875.

Zhang L, Zhang F, Melotto M, Yao J, He SY. 2017. Jasmonate signaling and manipulation by pathogens and insects. Journal of Experimental Botany **68**, 1371-1386.

Zhang PJ, Zheng SJ, van Loon JJA, Boland W, David A, Mumm R, Dicke M. 2009. Whiteflies interfere with indirect plant defense against spider mites in Lima bean. Proceedings of the National Academy of Sciences, USA **106**, 21202-21207.

Zhang PJ, Xu CX, Zhang JM, Lu YB, Wei JN, Liu YQ, David A, Boland W, Turlings TCJ. 2013a. Phloem-feeding whiteflies can fool their host plants, but not their parasitoids. Functional Ecology **27**, 1304-1312.

Zhang PJ, Broekgaarden C, Zheng SJ, Snoeren TAL, van Loon JJA, Gols R, Dicke M. 2013b. Jasmonate and ethylene signaling mediate whitefly-induced interference with indirect plant defense in *Arabidopsis thaliana*. New Phytologist **197**, 1291-1299.

Zhang SZ, Huang H, Shan HW, Zhang F, Wan FH, Liu TX. 2013. Defense against *Pieris rapae* in cabbage plants induced by *Bemisia tabaci* biotype B. Entomologia Experimentalis et Applicata **147**, 293-300.