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ORIGINAL PAPER

Host plants benefit from non-predatory effects of zoophytophagous predators against herbivores

Zhengyang Zhu[1,2,3] · Coline C. Jaworski[1,4] · Yulin Gao[5] · Zhigang Xu[3] · Junxiu Liu[1,2] · Ercheng Zhao[1] · Su Wang[1,2] · Nicolas Desneux[4] · Ning Di[1,2]

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Abstract

Zoophytophagous predators can induce plant defence responses through phytophagous feeding. Since the zoophytophagous bug *Orius sauteri* lays eggs into plant tissues, we hypothesised that its oviposition behaviour may also induce plant defence responses with a negative impact on subsequent herbivore attacks. Pre-inoculation of *O. sauteri* females on tomato plants significantly reduced the fitness and reproduction of *Frankliniella occidentalis*, which also preferred the non-inoculated plants in indoor micro-environments. In the field, *O. sauteri* pre-inoculation also caused reduced population growth of *F. occidentalis*. All these tendencies were weaker with male compared to female *O. sauteri* pre-inoculation. Next, a transcriptome analysis showed that the MAPK signalling pathway, the plant hormone signal transduction and plant-pathogen interaction of defence-related pathways were significantly enriched in plants inoculated with *O. sauteri* females compared to untreated plants. We showed that three key genes of the JA pathway, allene oxide synthase (*AOS*), jasmonate ZIM-domain 2 (*JAZ2*), and proteinase inhibitor 1 (*PI-1*), were upregulated. This is evidence of plant defence activation, the likely mechanism by which *O. sauteri* pre-inoculation (through feeding and oviposition activities) reduced *F. occidentalis* fitness in the laboratory and population densities by almost three times in a greenhouse experiment. This mechanism could be promoted in IPM strategies through the early introduction of zoophytophagous biocontrol agents activating crop plant defences to enhance biological pest control.

Keywords Defence responses · Fitness · Feeding · Oviposition behaviour · Biological control

Introduction

Over more than 400 million years of co-evolution between plants and herbivores, plants have developed a series of diverse and complex defence mechanisms against herbivore attacks (Hilker and Fatouros 2015; Schuman and Baldwin 2016; Erb and Reymond 2019; Pearse et al. 2020; Chen et al. 2023; Liu et al. 2023). Understanding the diverse ecological and biological interactions between host plants, herbivores, and natural enemies may help identify effective integrated pest management (IPM) strategies to improve pest management in biological pest control programmes (McCormick et al. 2012; Turlings and Erb 2018; Erb et al. 2021; Cruz-Miralles et al. 2022; Munawar et al. 2022; Sun et al. 2022; Depalo et al. 2022). Although plant–herbivore interactions have been intensively studied (Dicke and Baldwin 2010; McCormick et al. 2012; Mithöfer and Boland 2012; Bai et al. 2022), the effects of zoophytophagous predators on plant defences and their consequences on herbivore behaviour

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✉ Su Wang
wangsu@ipepbaafs.cn

✉ Ning Di
dining@ipepbaafs.cn

[1] Institute of Plant Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China

[2] Key Laboratory of Natural Enemies Insects, Ministry of Agriculture and Rural Affairs, Beijing 100122, China

[3] College of Agriculture, Nanjing Agricultural University, Nanjing 210095, China

[4] Université Côte d'Azur, INRAE, UMR ISA, 06000 Nice, France

[5] State Key Laboratory for Biology of Plant Disease and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China

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have only been studied recently (Bouagga et al. 2018a,b; Pérez-Hedo et al. 2021, 2022; Zhang et al. 2021). The feeding or oviposition behaviour of several zoophytophagous predators can activate the same plant defence mechanisms as those triggered by herbivores, affecting the fitness of herbivores in biological pest control (De Puysseleyr et al. 2010; Zhang et al. 2018, 2019; Cruz-Miralles et al. 2021; Pérez-Hedo et al. 2018, 2021, 2022).

Plant defence mechanisms are generally activated by phytohormones in the jasmonic acid (JA)-, salicylic acid (SA)-, abscisic acid (ABA)-, and ethylene (ET)-related pathways to regulate transcriptional responses (Wu and Baldwin 2010; Erb et al. 2012; Li et al. 2021; Ye et al. 2021; Ibanez et al. 2022). The JA pathway in particular plays a critical role in activating plant defences against herbivore attacks (Wang et al. 2019; Li et al. 2022). For example, the feeding and oviposition behaviour of *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) on plants significantly reduced the damage to plants by *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and by *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), through the activation of the JA and SA signalling pathways (De Puysseleyr et al. 2010; Bouagga et al. 2018b). Similarly, the zoophytophagous predator *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) simultaneously activated the JA and ABA pathways in tomato plants by puncturing leaves; this induced the release of a mixture of volatile organic compounds (VOCs) repellent to the herbivores *B. tabaci*, *F. occidentalis*, *Tetranychus urticae* Koch (Acari: Tetranychidae), and *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Pérez-Hedo et al. 2018, 2021, 2022). Finally, an inoculation of *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae) on tomato and sweet pepper plants reduced the performance of *F. occidentalis*, *T. urticae* and *B. tabaci* by activating the JA and ABA signalling pathways, inducing plant defence responses (Pappas et al. 2015; Zhang et al. 2018, 2019; Bouagga et al. 2018a). Recently, Pérez-Hedo et al. (2022) comprehensively reported that zoophytophagous predators, such as Miridae, Anthocoridae, Pentatomidae, and predatory mites, can induce plant defences, which in turn can enhance pest control efficiency, although the differences in plant-mediated effects of feeding versus oviposition are not well understood yet.

Many species of the zoophytophagous predator *Orius* genus (Hemiptera: Anthocoridae) have been extensively studied for their role as natural enemies controlling agricultural pests (e.g. Desneux et al. 2006; Ragsdale et al. 2011). The flower bug *O. sauteri* (Poppius) (Hemiptera: Anthocoridae) is widely used as an efficient biological control agent against thrips, whiteflies, aphids, spider mites, and eggs and young larvae of lepidopteran pests; it also naturally occurs in cultivated open fields in China (Zhao et al. 2017; Wang et al. 2018; Di et al. 2022; Mouden et al. 2017; Reitz et al. 2020). Although *O. sauteri* has a wide prey breadth, it prefers to prey upon *F. occidentalis* over whiteflies, spider mites, and aphids (Xu and Enkegaard 2009; Wang et al. 2014; Di et al. 2022). For this reason, *O. sauteri* has been used as the main biological control agent against *F. occidentalis*, which is one of the most devastating pests in horticultural crops, causing heavy yield losses worldwide (Mouden et al. 2017; Reitz et al. 2020).

In addition to feeding on prey, *Orius* species require plant resources for their development and reproduction (De Puysseleyr et al. 2010; Ding et al. 2021; Ren et al. 2022). Plants not only act as a source of water and nutrients but also serve as oviposition substrates for *Orius* species (Lundgren et al. 2008; De Puysseleyr et al. 2010). *Orius* species insert their eggs into plant tissues to obtain nutrients and the environmental conditions that allow offspring to survive for several days without additional resources (Di et al. 2022). In *O. sauteri*, eggs are almost entirely laid within plant tissues – with only a small part of the egg operculum exposed – causing obvious physical and physiological damage to the plant tissues (De Puysseleyr et al. 2010; Di et al. 2022). Hence, this special oviposition behaviour of *O. sauteri* may cause the same induced plant defence responses as those stimulated via phytophagous feeding by herbivores and zoophytophagous predators. Such plant defence priming could negatively affect the fitness of herbivores and improve pest management, similar to other zoophytophagous predators (De Puysseleyr et al. 2010; Bouagga et al. 2018a; Cruz-Miralles et al. 2021; Dahmane et al. 2022; Pérez-Hedo et al. 2022).

In this study, we measured the effects of pre-inoculating tomato plants with *O. sauteri* females (feeding and oviposition) or males (feeding only) on the performance of *F. occidentalis* in the laboratory and in greenhouses. In addition, a transcriptomic analysis was conducted to identify the key genes associated with plant defence induction and to measure their relative expression levels through time in plants inoculated with either female or male *O. sauteri*. This study aimed to establish a theoretical understanding of the ecological and biological interactions between the zoophytophagous predator *O. sauteri* and the pest *F. occidentalis* to enhance pest control efficiency.

Materials and methods

Plants and insects

Plants

Tomato *Solanum lycopersicum* L. plants were used as experimental materials. Tomato seeds (CV. Jiaxin M5020) were sown in a 24-hole seed tray (36.5 × 23.0 × 5.5 cm) filled with growth medium of a mixture of peat soil (Pindstrup Mosebrug A/S, 0 ~ 10 mm, Denmark) and vermiculite (3: 1, V:

V). Then, each tomato seedling was transplanted at the two-leaf stage into a plastic flowerpot (8.0 × 8.0 × 10.0 cm) filled with growth medium of a mixture of peat soil, vermiculite, and pearlite (3:1:1, *V*:*V*:*V*). Tomato plants were grown under controlled conditions at day/night temperature of (26 ± 2)/(18 ± 2) °C, relative humidity (RH) of 50 ± 5%, photoperiod of 16/8 h (day/night), and 10,000 Lux fluorescent light in an insect-free artificial growth chamber located at the Institute of Plant Protection (BIPP), Beijing Academy of Agriculture and Forestry Sciences (BAAFS, Haidian District, Beijing, China). The tomato plants were grown to the five-leaf stage for subsequent experiments.

Insects

An initial adult colony of *O. sauteri* was collected from maize fields in Langfang, Hebei Province, China, during the summer of 2018, and the colony was re-supplied with new individuals from this area for rejuvenation every year. A colony was subsequently established and reared using hyacinth bean *Lablab purpureus* (L.) Sweet pods (Fabales: Fabaceae). *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) eggs were used as food sources, as previously described (Di et al. 2022). A colony of *F. occidentalis* was obtained from the Institute of Plant Protection (IPP), Chinese Academy of Agricultural Sciences (CAAS, Haidian District, Beijing, China). Adults and nymphs of *F. occidentalis* were reared on hyacinth bean pods similar to *O. sauteri* (Di et al. 2022).

Fitness of *F. occidentalis* on tomato plants pre-inoculated with *O. sauteri*

Survival and fecundity of *F. occidentalis* on tomato plants pre-inoculated with *O. sauteri*

Tomato plants with five true leaves were used to measure the effect of *O. sauteri* pre-inoculation on the survival and fecundity of *F. occidentalis* on tomato plants. The third leaf from the bottom of each tomato plant was enclosed in a leaf cage (Di et al. 2022). Three treatments were used: (1) pre-inoculation of *O. sauteri* females (OsF), (2) pre-inoculation of *O. sauteri* males (OsM), and (3) control, no pre-inoculation. In (1) and (2), three mated *O. sauteri* females versus three males aged 3–5 days after emergence were inoculated into the leaf cage and were removed 24 h later. Then, 20 mated females of *F. occidentalis* aged 2–3 days after emergence were infested into each leaf cage. The number of surviving *F. occidentalis* adults was counted and recorded 24 and 48 h later, after which *F. occidentalis* adults were removed. 120 h after *F. occidentalis* infestation, the number of *F. occidentalis* F1 nymphs was recorded as a measure of fecundity. This experiment was conducted in an artificial growth chamber under the same environmental conditions as above ("Plants" section). There were 12 replicates per treatment in this experiment.

Preference of *F. occidentalis* for plants pre-inoculated with *O. sauteri* versus control plants

In a preference choice test for *F. occidentalis*, tomato plants and predators were treated as described above ("Survival and fecundity of F. occidentalis on tomato plants pre-inoculated with O. sauteri" section), except for the leaf cages. The leaf cage in this experiment was made of two parts, a nylon yarn net part (pore size: 150 μm; size: 15.0 cm × 13.0 cm) and a zip lock bag part connected to the base of the third leaf. Predators were removed 24 h after inoculation. Tomato plants from the control and OsF, control and OsM, and OsF and OsM groups were used in paired choice tests. Each plant from a pair was placed at one side of a choice system (ZL201922103394.4), which was made of two nylon yarn net bags (pore size: 150 μm; size: 30.0 cm × 25.0 cm), each covering a whole plant, and a glass T-tube fixed in the middle of the two bags. One *F. occidentalis* mated female aged 2–5 days after emergence was placed at the middle of the *T*-tube, and was left to choose between the two plants for 5 min. If it spent more than 15 s in one side, it was considered as a valid selection, while no choice during 5 min was considered an invalid selection; in this case, it was recorded as 'no-choice' and excluded from data analysis (Guo et al. 2019). After recording three individuals, the plant positions were swapped between the left and right side to avoid any spatial effect on choices and three new individuals were tested. This was repeated with a new plant combination. Each individual was tested only once. 48 individuals were tested for each combination.

Population dynamics of *F. occidentalis* on tomato plants pre-inoculated with *O. sauteri*

The experiments were conducted in a greenhouse at the Wangjiayuan Seven-Star Apple Professional Cooperative (Changping District, Beijing, China) in October and November 2020 and 2021. Tomato plants at the stage five true leaves were simultaneously transplanted into three greenhouses. In each greenhouse, three groups of six to ten tomato plants in two rows were established and randomly assigned to one of the three treatments (control, OsF, OsM). In 2020, the number of tomato plants per treatment were 9, 6, 9 in blocks A, B, C for control; 8, 10, 8 in blocks A, B, C for OsF; and 6, 6, 6 in blocks A, B, C for OsM. This asymmetric design due to logistic constraints was optimised in 2021, leading to 8, 10, 10 plants in blocks A, B, C for each treatment. Seven days later, plants were inoculated with *O. sauteri* as described above ("Preference of F. occidentalis for plants pre-inoculated with O. sauteri versus control plants"

section), except that natural enemies were inoculated again every 14 days after the first inoculation at a rate of six individuals per leaf cage. This was to ensure a persistent inoculation effect of the predators on herbivores. Subsequently, 20 mated *F. occidentalis* females were released per tomato plant. The temperature and lighting conditions during the experiment followed the local natural sunlight environment under shelter. The number of adults and nymphs of *F. occidentalis* in all leaves of six to ten tomato plants in each treatment in each block was then recorded every 6–8 days for 1.5 months. The experiments were ended when the population dynamics of *F. occidentalis* decreased below the initial infestation density.

Oviposition behaviour of *O. sauteri*

Tomato plants with five true leaves were used to measure the oviposition of *O. sauteri* on tomato plants in the laboratory. A leaf cage (Di et al. 2022) was placed on the third leaf from the bottom of the tomato plants, and three *O. sauteri* mated females aged 3–5 days after emergence were inoculated into the leaf cage. The number of eggs laid was recorded 6, 12, 24, 48, 72, and 96 h after *O. sauteri* inoculation in 12 leaf cage replicates each, using a digital microscope (VHX-6000, Keyence, Japan). The shape of eggs was photographed on hyacinth bean pods using a cryogenic scanning electron microscope (Regulus 8100, Hitachi, Japan).

Transcriptome sequencing analysis of tomato plants pre-inoculated with *O. sauteri*

In this experiment, tomato plants were treated as described above ("Preference of F. occidentalis for plants pre-inoculated with O. sauteri versus control plants" section), and predators were removed from leaf cages 24 h later. Transcriptome sequencing analysis was performed using four biological replicates for each treatment. At this time, the third leaf from the bottom of each tomato plant was cut off, immediately frozen in liquid nitrogen, and then stored at − 80 °C for future use. Total RNA was extracted from each tomato leaf sample using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The RNA concentration was measured using a NanoDrop ONE spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) at 260 nm. RNA-seq library construction, RNA-seq, and RNA seq data analysis were conducted following Biomarker (Beijing, China) on an Illumina Xten HiSeq platform (Chen et al. 2023). The tomato reference genome *Solanum lycopersicum*_ITAG4.0 (https://solgenomics.net/organism/Solanum_lycopersicum/genome) was used. The relative expression abundances of RNA-seq were estimated based on the number of fragments per kilobase of transcript per million reads mapped (FPKM). Finally, we used the DESeq R package to identify differentially expressed genes (DEGs) between plants inoculated with *O. sauteri* and control plants using the following criteria: fold change in expression level ≥ 1.5 or ≤ 0.67 and false discovery rate < 0.05 (Chen et al. 2023).

Relative expression of key genes in the JA pathways in tomato plants inoculated with *O. sauteri*

Because the JA pathway was found to be significantly enriched, we focussed on relative expression levels of key genes in the JA pathway. In this experiment, tomato plants were treated as described in "Preference of F. occidentalis for plants pre-inoculated with O. sauteri versus control plants" section, except for the duration of predator inoculation. The sampling timepoints were 6, 12, 24, 48, 72, and 96 h after inoculation. Five tomato plants were collected from each treatment at each timepoint. Total RNA was extracted, and we quantified its concentration in tomato leaves using the TRIzol reagent method described above ("Transcriptome sequencing analysis of tomato plants pre-inoculated with O. sauteri" section). Moreover, 1.0 µg of total RNA was treated by adding PrimeScript™ RT reagent Kit with gDNA Eraser (Perfect Real Time) (Takara, Kusatsu, Shiga, Japan) to obtain the first-strand cDNA. The synthesised cDNA was immediately stored at -20 °C for subsequent use. The relative expression levels of the three genes allene oxide synthase (AOS), jasmonate ZIM-domain 2 (JAZ2), and proteinase inhibitor 1 (PI-1), were quantified at each timepoint for each treatment using quantitative real-time polymerase chain reaction (qRT-PCR) on ABI QuantStudio™ 7 Flex Real-Time PCR System (Applied Biosystems, Hercules, CA, USA) using TB Green® Premix EX Taq™ II (Tli RNaseH Plus) master mix (Takara, Kusatsu, Shiga, Japan). The optimised qRT-PCR programme consisted of an initial denaturation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min; finally, melting curves were plotted at 95 °C for 15 s. Melting curves were analysed to determine the purity of the qRT-PCR products. qRT-PCR was performed on three technical replicates for each of the five biological replicates for each treatment and time point. The transcript levels of AOS, JAZ2, and PI-1 were expressed as normalised transcript abundances using Actin as an internal reference gene. The relative expression of AOS, JAZ2, and PI-1 were calculated according to the $2^{-\Delta\Delta Ct}$ method. qRT-PCR primers were designed using the Primer 3+software (https://www.primer3plus.com) based on *AOS*, *JAZ2*, and *PI-1* sequences of tomatoes in the NCBI database (Table S1).

Statistical analyses

All statistical analyses were performed using R Core Team (2023) version 4.3.1. The effect of the pre-inoculation treatment (control, *O. sauteri* female, *O. sauteri* male) in interaction with time (24 h or 48 h) on the survival rate of *F. occidentalis* was analysed using a generalised linear model (GLM) with a binomial error distribution (function 'glm'; R Core Team, 2023). The effect of the pre-inoculation treatment on the fecundity (number of *F*1 nymphs) of *F. occidentalis* was analysed using a linear model (LM). The effect of the pre-inoculation treatment on *F. occidentalis* field densities each year (2020 and 2021) was tested using independent generalised linear mixed models (GLMMs) with a negative binomial error distribution (function 'glmer.nb'; R library 'lme4'; Bates et al. 2015). Each year, the pre-inoculation treatment (control, *O. sauteri* female, *O. sauteri* male) was implemented as a fixed effect in interaction with the covariate sampling date, while the plant nested in the block were implemented as random effects. The effect of the inoculation treatment (control, *O. sauteri* female or *O. sauteri* male) on the expression level of three genes of the JA pathway (*AOS*, *JAZ2* and *PI-1*) was analysed using independent linear mixed models (LMMs) with the treatment in interaction with the time since inoculation as fixed effects, and the replicate as random effect. Data were previously square-root transformed for *JAZ2* and *PI-1*.

The normality and homogeneity of residuals was checked a posteriori for each test in the best model using the function 'plot(simulateResiduals())' (R library 'DHARMa'; Hartig 2022). The significance of interaction terms and single fixed effects were analysed in each test using an ANOVA with a χ^2 test for GLMs, GLMMs and LMMs, and a Fisher test for LMs. When significant effects were found, post hoc mean comparisons were performed using the 'emmeans' function (R library 'emmeans'; Lenth 2023). The choice test for *F. occidentalis* was analysed using a χ^2 goodness-of-fit test using SPSS 25.0 (IBM, Armonk, NY, USA) based on a null model where the treated and control plants were selected with equal frequency. Individuals which did not make a choice were excluded from the statistical analysis.

Results

Fitness of *F. occidentalis* on tomato plants inoculated with *O. sauteri*

Survival and fecundity of *F. occidentalis* on tomato plants pre-inoculated with *O. sauteri* in the laboratory

The pre-inoculation treatment caused a reduction in *F. occidentalis* survival (Dev = 32.2, df = 2, $P < 0.001$; Fig. 1a) and the survival rate was lower after 48 h compared to 24 h (Dev = 10.1, df = 1, $P = 0.0014$). However, the effect of the pre-inoculation treatment did not vary across time (non-significant treatment by time interaction: Dev = 1.24, df = 2, $P = 0.54$). The reduced survival was observed in the *O. sauteri* female pre-inoculation treatment only, which was significantly lower compared to the control (estimate ± SE: − 0.798 ± 0.17, $P < 0.001$) and compared to the *O. sauteri* male pre-inoculation treatment (− 0.765 ± 0.17, $P < 0.001$), while the *O. sauteri* male pre-inoculation treatment was not different from the control (− 0.0329 ± 0.181, $P = 0.98$). The pre-inoculation treatment also caused a reduction in *F. occidentalis* fecundity ($F_{2,33} = 39.0$, $P < 0.001$; Fig. 1b): both *O. sauteri* female (estimate ± SE: − 22.6 ± 2.6, $P < 0.001$) and male (− 10.2 ± 2.6, $P < 0.001$) pre-inoculations caused a significant reduction in F1 nymphs numbers compared to the control, but the female pre-inoculation caused a stronger reduction compared to the male pre-inoculation (− 12.3 ± 2.6, $P < 0.001$).

Preference of *F. occidentalis* between plants pre-inoculated with *O. sauteri* and control plants in a laboratory choice test

In the choice test, *F. occidentalis* females showed a significant preference for control tomato plants over tomato plants pre-inoculated with *O. sauteri* females (OsF) ($N = 42$, no choice = 5, $\chi^2 = 12.3$, $P < 0.001$; Fig. 1c). There was no significant preference between control plants and tomato plants pre-inoculated with *O. sauteri* males (OsM) ($N = 42$, no choice = 5, $\chi^2 = 2.38$, $P = 0.12$), although they tended to choose OsM less often than control plants. Similarly, *F. occidentalis* chose OsM more frequently than OsF plants, but there was no significant difference ($N = 42$, no choice = 5, $\chi^2 = 1.52$, $P = 0.22$).

Field population dynamics of *F. occidentalis* on tomato plants pre-inoculated with *O. sauteri*

The population of *F. occidentalis* in the field first increased and then decreased in both years (Fig. 1d, e). The pre-inoculation treatment significantly reduced the abundances of *F. occidentalis* in interaction with the sampling date in 2020 ($\chi^2 = 26.3$, df = 10, $P = 0.0034$; Fig. 1d). *Orius sauteri* female pre-inoculation prevented *F. occidentalis* populations to reach a peak in early November, while the densities at the peak in the *O. sauteri* male pre-inoculation were intermediate between the control and the female pre-inoculation treatment (Table S3; Fig. 1d). In 2021, the pre-inoculation treatment also significantly reduced the densities of *F. occidentalis* ($\chi^2 = 23.0$, df = 2, $P < 0.001$; Fig. 1e), but independently of the sampling date (non-significant treatment * date interaction: $\chi^2 = 11.5$, df = 10, $P = 0.32$). Both *O. sauteri* female (estimate ± SE:

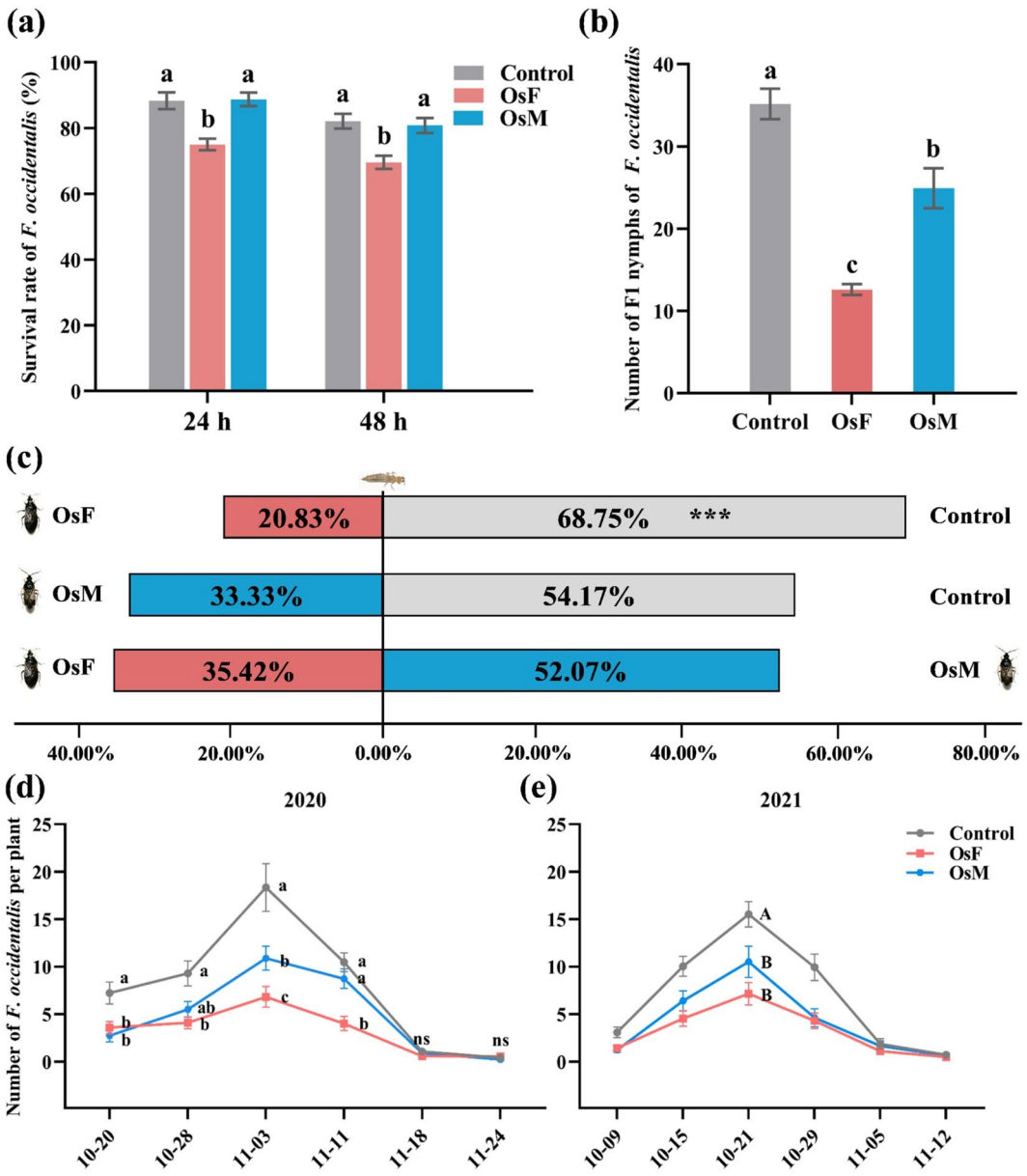

Fig. 1 Fitness and preference of *Frankliniella occidentalis* on tomato plants pre-inoculated with *Orius sauteri.* (**a**) Survival rate of *F. occidentalis* on tomato plants inoculated with *O. sauteri.* (**b**) Fecundity (F1 nymph number) of *F. occidentalis* on tomato plants pre-inoculated with *O. sauteri*. (**c**) Choice of *F. occidentalis* on tomatoes pre-inoculated with *O. sauteri.* (**d**, **e**) Field population dynamics of *F. occidentalis* on tomato plants pre-inoculated with *Orius sauteri.* OsF: tomato plants inoculated with *O. sauteri* female adults (feeding and oviposition); OsM: tomato plants inoculated with *O. sauteri* male adults (feeding only); control: tomato plants not inoculated with *O. sauteri*. Different lowercase letters indicate significant differences at $P < 0.05$ (Tukey's test). Data are presented as mean ± SE in (**a**, **b**, **d**, **e**). (**a**, **b**) $N = 12$ replicates; (**c**) $N = 48$ individuals tested per combination; (**d**, **e**) $N = 6$ to 10 replicates per sampling date per block with 3 blocks. Different lowercase letters in (**a**, **b**, **d**) indicate significant differences between treatments (for each time in (**a**) and each date in (**d**); see Table S3). *** in (**c**) indicates a significant difference at $P < 0.001$. Different uppercase letters in (**e**) indicate significant differences between treatments, independently of the sampling date

-0.802 ± 0.168, $P < 0.001$) and male (-0.664 ± 0.168, $P < 0.001$) pre-inoculations led to reduced *F. occidentalis* densities compared to the control, but the *O. sauteri* female pre-inoculation effect was not different from the male one (-0.138 ± 0.172, $P = 0.70$).

Oviposition behaviour of *O. sauteri* on tomato plants

Eggs of *O. sauteri* were laid inside the plant tissues (Fig. 2a, c) with only the operculum exposed (Fig. 2b). The number of eggs laid by *O. sauteri* on tomato plants increased linearly

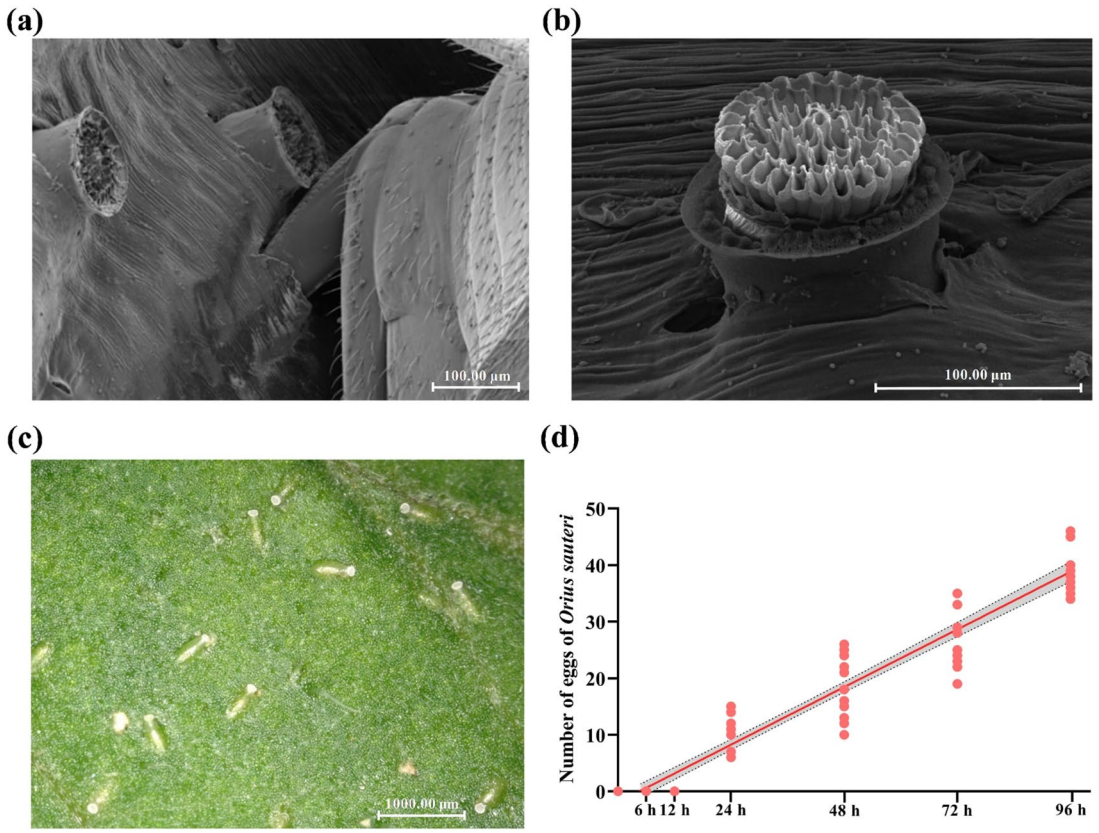

Fig. 2 Oviposition behaviour of *Orius sauteri* on plants. (**a**) The ovipositor of *O. sauteri* on the hyacinth bean pods, which was captured at 220 × magnification (Cryo-SEM Regulus 8100, Hitachi, Japan). (**b**) The operculum of an egg of *O. sauteri* on the hyacinth bean pods, which was captured at 500 × magnification a Cryo-SEM (Regulus 8100, Hitachi, Japan). (**c**) Eggs of *O. sauteri* on the tomato leaves, captured at 50 × magnification under a digital microscope (VHX-6000, Keyence, Japan); (**d**) egg number of *O. sauteri* on tomato plants at different timepoints

with time ($R^2 = 0.9352$). No eggs were laid before 24 h after the inoculation of *O. sauteri*, but a increase from 10.6 ± 0.9 at 24 h to 40.0 ± 1.6 at 96 h was recorded (Fig. 2d).

Transcriptome responses in tomato plants inoculated with *O. sauteri*

Differentially expressed genes (DEGs) after *O. sauteri* inoculation in tomato plants were identified to understand plant response to *O. sauteri*. The detailed information on RNA-seq data and mapping is summarised in Table S2. A total of 35,096 transcripts were detected across all samples (Data S1). 761 DEGs were identified, including 726 upregulated and 35 downregulated in OsF compared to control plants, 44 upregulated and 0 downregulated in OsM compared to control plants, and 48 upregulated and 1 downregulated in OsF compared to OsM plants, respectively (Fig. S1a, b, c; Data S2). The DEGs in control versus OsF, control versus OsM, and OsF versus OsM comparisons were assigned to 72, 16, and 19 KEGG pathways, respectively, and the top 20, 16, and 19 pathways for each pairwise comparison are shown in Fig. 3 and Table S3. Of these, the mitogen-activated protein kinase (MAPK) signalling pathway-plant, plant hormone signal transduction, and plant-pathogen interaction were the only three pathways that were significantly enriched in OsF plants compared to control plants, and the relative abundance of their DEGs showed the highest expression in the OsF treatment, while there were no significantly enriched pathways in OsM plants compared to control plants or to OsF plants (Fig. S2).

Relative expression levels of key genes of the JA pathway in tomato plants inoculated with *O. sauteri*

The three key genes of the JA pathway – *AOS*, *JAZ2* and *PI-1* – all had a bell-shaped expression level pattern, where expression levels first increased from 6 h to 24 or 48 h after inoculation, and then decreased until 96 h. For all three genes, the inoculation treatment in interaction with the time since inoculation significantly affected the expression level (*AOS*: $\chi^2 = 69.5$, df = 10, $P < 0.001$; *JAZ2*: $\chi^2 = 184$, df = 10, $P < 0.001$; *PI-1*: $\chi^2 = 182$, df = 10,

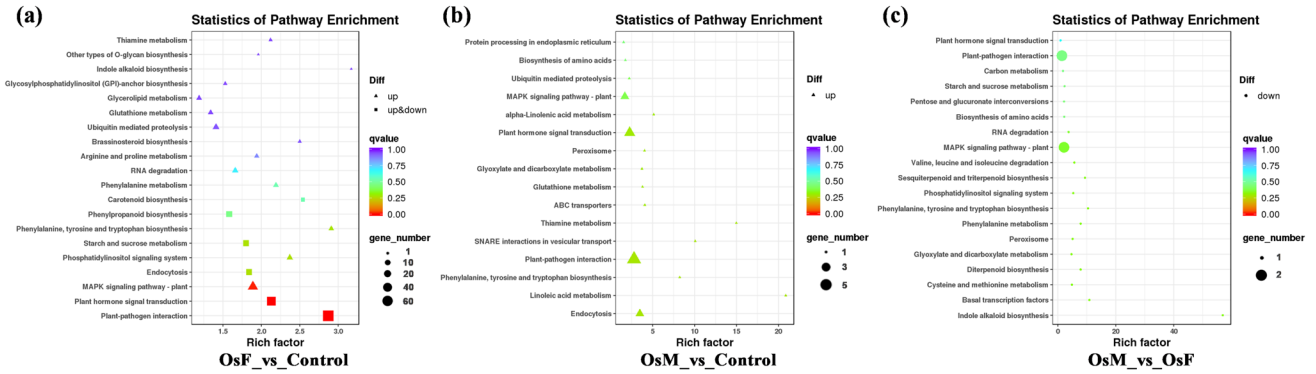

Fig. 3 Kyoto Encyclopedia of genes and genomes (KEGG) pathway enrichment analysis of differentially expressed genes (DEGs) in tomato plants inoculated with *Orius sauteri*. The top 20 KEGG pathway enrichment analysis of DEGs under the **a** OsF_vs_Control, **b** OsM_vs_Control, and **c** OsM_vs_OsF comparisons. OsF: tomato plants inoculated with *O. sauteri* female adults (feeding and oviposition); OsM: tomato plants inoculated with *O. sauteri* male adults (feeding only); control: tomato plants not inoculated with *O. sauteri*. The colour of the node represents a different KEGG term. The size of the node represents the term enrichment significance. *Y* axis, different KEGG pathway; *X* axis, rich factor. The rich factor represents the ratio of the proportion of genes annotated to the pathway amongst DEGs to the proportion of genes annotated to the pathway amongst all genes. The colour and size of the node represents the pathway enrichment significance (qvalue < 0.05 = significant enrichment) and the number of DEGs enriched in this pathway, respectively

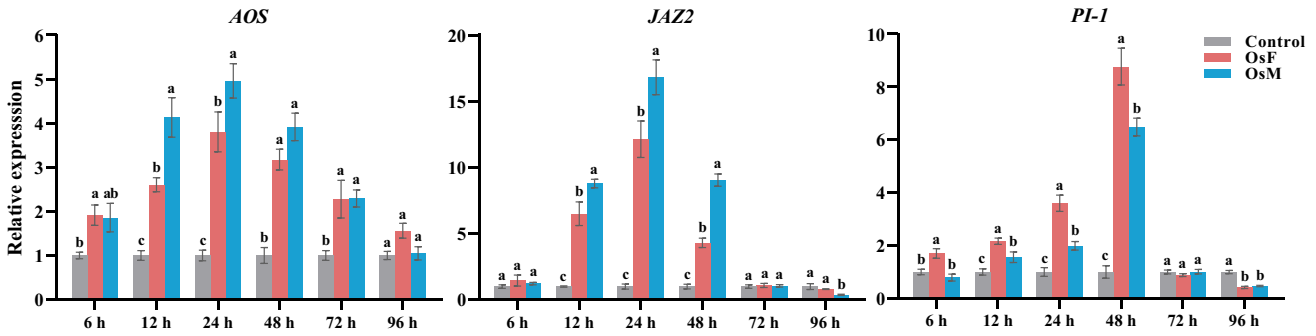

Fig. 4 Relative expression of key genes (mean ± SE) of the JA pathway in tomato leaves up to 96 h after inoculation with *Orius sauteri* female and male adults. *AOS*, allene oxide synthase; *JAZ2*, Jasmonate ZIM-domain 2; *PI-1*, proteinase inhibitor 1. OsF: tomato plants inoculated with *O. sauteri* female adults (feeding and oviposition); OsM: tomato plants inoculated with *O. sauteri* male adults (feeding only); Control: tomato plants not inoculated with *O. sauteri*. Different lowercase letters indicate significant differences between treatments for each time and each gene (see Tables S4–6)

$P < 0.001$; Fig. 4, Table S4–6). The expression of AOS was maximal 24 h after inoculation in the OsF and OsM treatments, and was higher in the OsF treatment compared to the OsM treatment from 12 to 24 h after inoculation, while the expression level in the OsF and OsM treatments was higher than in control from 6 to 72 h after inoculation. Similarly, the expression of *JAZ2* was highest in the OsF and OsM treatments 24 h after inoculation, but was higher in the OsM than in the OsF treatment and in the OsF treatment than in the control from 12 to 48 h after inoculation. Finally, the expression level of *PI-1* was highest 48 h after inoculation, and it was higher in the OsF than in the OsM treatment from 6 to 48 h after inoculation, while it was higher in the OsM treatment than in the control from 12 to 48 h after inoculation. Also, 96 h after inoculation, the expression level of *PI-1* was lower in both the OsF and OsM treatments compared to the control.

Discussion

Orius sauteri has been widely used as a biological control agent in China against thrips (Mouden et al. 2017; Reitz et al. 2022; Di et al. 2022). However, whether its oviposition behaviour—the insertion of eggs into plant tissues—activates plant defences has rarely been studied. Here, we found that the pre-inoculation of both *O. sauteri* females and males on tomato plants reduced the fitness of *F. occidentalis*, both in the laboratory (reduced survival and fecundity) and in greenhouse conditions (reduced population densities)

with a bigger effect of females pre-inoculation. This is likely related to *O. sauteri* oviposition behaviour compared to the feeding-only activity of male adults. The transcriptome analysis confirmed this hypothesis, indicating that only female pre-inoculation activated the MAPK signalling pathway, plant hormone signal transduction, and plant-pathogen interaction of defence-related KEGG pathways. This highlights the mechanism at stake, where *O. sauteri* oviposition activity induces plant defence, in turn reducing *F. occidentalis* fitness.

The pre-inoculation of *O. sauteri* females significantly reduced the survival rate of *F. occidentalis*, whereas males did not. Pre-inoculation of both *O. sauteri* females and males significantly lowered the F1 nymph number of *F. occidentalis*, although the effect from females was larger. Similarly, Pappas et al. (2015) reported that tomato plants punctured with *M. pygmaeus*, a zoophytophagous predator that depends on plants for survival, significantly reduced the survival rate and egg number of *T. urticae* pests. Compared to the control plants, *M. pygmaeus*-punctured sweet pepper plants significantly reduced the survival rate of *T. urticae* and reproduction of *F. occidentalis* and *T. urticae* (Zhang et al. 2018), which is consistent with the findings of our study.

Here we found that female but not male pre-inoculation treatments significantly repelled *F. occidentalis* females. This may be due to the induced plant defence response caused by *O. sauteri* oviposition activity, cascading to the emission of associated volatile organic compounds which may repel *F. occidentalis*. Indeed, Bouagga et al. (2018b) found that sweet pepper plants punctured by *O. laevigatus* were significantly less appealing to *F. occidentalis* and *B. tabaci* because of the release of terpenoids and green leaf volatiles through the activation of the JA and SA pathways. Similarly, De Puysseleyr et al. (2010) specifically found that the oviposition behaviour but not plant feeding of *O. laevigatus* females reduced damage caused by *F. occidentalis* on tomato plants. The reduced attractiveness of host plants to pests after oviposition in plant tissues has also been demonstrated for mirid bugs, which activated the JA and ABA plant defence pathways both via oviposition and plant feeding (Pérez-Hedo et al. 2018, 2021; Bouagga et al. 2018a; Zhang et al. 2019).

The induction of plant defences through *O. sauteri* oviposition could have biocontrol implications through bottom-up effects regulating pest populations, but also through top-down effects if plant defence priming affects the attraction of other natural enemies (Han et al. 2022; Liu et al. 2022). Here, we found that tomato plants pre-inoculated with *O. sauteri* females had significantly lower *F. occidentalis* population densities compared to plants pre-inoculated with *O. sauteri* males and to control plants at the population density peak in 2020 and 2021 in the field. Similarly, Dahmane et al. (2022) found that compared to the control, citrus plants pre-infested with *Pilophorus clavatus* (Hemiptera: Miridae) significantly reduced numbers of *T. urticae* from seven day after *T. urticae* release. The differences between females and male treatments observed here may be due to the oviposition behaviour of *O. sauteri* females and similar to results from the laboratory experiment of *F. occidentalis* performance. In particular, key genes of the JA signalling pathway, including *PI-1*, were upregulated by *O. sauteri* female inoculation. Protease inhibitors are important end products of the JA pathway, and may be the direct cause of *F. occidentalis* reduced fitness (Takahashi et al. 2007; Zhang and Zhang 2022).

In total, 35,096 transcripts were detected in the different *O. sauteri* treatments. Genes involved in plant defence mechanisms were significantly upregulated in tomato plants inoculated with female adults of *O. sauteri*, which is consistent with an accrued role of oviposition behaviour in plant defence induction. The 726 upregulated genes were found in OsF plants compared to control plants, while only 44 were upregulated in OsM plans compared to control plants. The three upregulated KEGG pathways in OsF plants—MAPK signalling, plant-plant hormone signal transduction, and plant-pathogen interaction—regulate and activate plant defence mechanisms together against herbivore and pathogen attacks (Takahashi et al. 2007; Wu et al. 2007; Hettenhausen et al. 2015; Zhang and Zhang 2022). Following plant attack by herbivores, the MAPK signalling pathway is activated, which in turn alters the levels of the phytohormones JA and ET, reshaping the transcriptome, and thus, activating defence responses against herbivore attacks (Hettenhausen et al. 2015; Meza-Canales et al. 2017; Sözen et al. 2020). Investigating the links between MAPK and JA activation in the current system should help further understand plant defence induction, and the consequences on reduced pest fitness.

Previous studies have shown that plants punctured with zoophytophagous predators induced plant defence responses by activating plant hormone signalling pathways such as JA, SA, and ABA, thus reducing the fitness of pests (Pérez-Hedo et al. 2021, 2022). In this study, only the JA signalling pathway was significantly upregulated by *O. sauteri* oviposition behaviour. While the number of eggs of *O. sauteri* inserted into tomato plant tissues increased linearly with time, the relative expression levels of AOS, JAZ2, and PI-1, i.e. key genes of the JA pathway, first increased until 24–48 h and then decreased with an increase in the duration of *O. sauteri* inoculation. Such short-time, bell-shaped gene expression change is evidence for the induction of plant defences, which happens over the short-term following plant tissue damage (Guo et al. 2019). It would be interesting to further investigate how the intensity of egg laying (number of eggs) matches the intensity of plant defence induction.

In the present study, we found that only *O. sauteri* female oviposition behaviour induced a significant plant defence response via the JA signalling pathway, although male feeding-only behaviour also induced a weaker plant defence response. This indirect, plant-mediated interaction by *O. sauteri* females on *F. occidentalis* could be exploited for the integrated pest management of *F. occidentalis*. Beyond the release of mass-reared natural enemies, other ecological interactions between plants, pests and natural enemies such as bottom-up effects through the induction of plant defences and alteration of plant perception by pests can offer interesting avenues to facilitate the regulation of pest populations. Here we showed that *O. sauteri* oviposition behaviour reduces *F. occidentalis* fitness. To develop new IPM tools, it would be useful to investigate the relative effects of plant feeding and oviposition by natural enemies versus by pests in other crop plants, to highlight processes beneficial to pest control.

Author contributions

ND, ND, and SW designed the assay; ZZ, YG, JL and EZ conducted the experiments; ZZ, ND, CCJ and SW analysed the data; ZZ, ZX, SW, CCJ and ND wrote the manuscript. All authors read and approved the manuscript.

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

Declarations

Competing interests The authors declare no competing interests.

Conflict of interest The authors declare no conflict of interest. Nicolas Desneux serves as Editor-in-Chief of Journal of Pest Science and was not involved in the review process and decisions related to this manuscript.

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