

# Supporting Information

Zhang et al. 10.1073/pnas.0907890106

## SI Text

**Effects of *B. tabaci* Infestation or SA Treatment of Lima Bean Plants on the Performance of Spider Mites.** To determine the effects of *B. tabaci* infestation or exogenous SA application on the performance of *T. urticae*, we assessed the oviposition rate of female *T. urticae*. In the whitefly treatment, Lima bean plants were infested with *B. tabaci* (at a density of 50 adults/leaf) for 7 days in a cage (30 × 40 × 60 cm), and control plants were placed in cages and left uninfested for 7 days. In the SA treatment, plants were sprayed with 1.25 mL/leaf of an SA solution in water (at a dose of 1 mM, containing 0.1% Tween 20) and incubated for 3 days, and plants kept under the same conditions were sprayed with 1.25 mL/leaf of water (containing 0.1% Tween 20) and were regarded as controls. Thereafter, three young-adult female *T. urticae* 10 days after hatching from the same cohort of eggs were transferred onto each leaf disc from the treated and control plant, respectively. After 3 days, the number of eggs laid by the females on control and treated leaves was counted. The experiment was replicated 10 times for both control and treatment.

**Effects of *B. tabaci* Infestation or SA Treatment of Lima Bean Plants on the Settling Behavior of Spider Mites.** Plants previously infested on one leaf by 50 *B. tabaci* adults, or sprayed on one leaf with 1.25 mL of 1.0 mM SA were used. For the bioassay experiment we used the systemic leaf that was not itself infested with *B. tabaci* or treated with SA. In the whitefly treatment, the herbivores were confined on one leaf by enclosing them in a clip cage (diameter 9.5 cm) on one of the two primary leaves. After 7 days of *B. tabaci* feeding, the leaf free of *B. tabaci* infestation was used for the bioassay. Control plants received a clip cage and were left uninfested. In the SA treatment, before SA (1 mM) was applied, one of the two leaves was covered with a plastic bag. Immediately after SA application to the other leaf, the plastic bag was removed. After 3 days, the leaf free of SA of the SA-treated plant was used in the bioassay. Plants left uninfested were regarded as controls. In this experiment, spider mites were offered two leaf discs (diameter 1.0 cm): one from a *B. tabaci*-infested plant or from an SA-treated plant and one from a control plant. Leaf discs were placed in a Petri dish with moist cotton wool, and were connected by a T-shaped bridge (2.5 cm wide) that was cut from Parafilm. The position of the discs was alternated between replicates. We then released six adult female *T. urticae* at the base of the T-bridge. After 24 h we counted the number of the spider mite adults on each disc of the two-choice setup.

**Quantification of Endogenous SA.** The quantification of endogenous SA was done as described in the main article. This was done for plants infested with 10 spider mites per leaf or with 10 spider mites plus 50 whiteflies per leaf after 12 h, 3 days, or 7 days since infestation.

**Quantitative Real-Time PCR.** The method as described in the main article was followed to quantify the transcript levels of *PIOS* (*Phaseolus lunatus ocimene synthase*, GenBank accession EU194553) relative to *PLACT1* (housekeeping gene, GenBank accession DQ159907) in plants infested with 10 spider mites per

leaf or with 10 spider mites plus 50 whiteflies per leaf after 12 h, 3 days, or 7 days since infestation.

**Statistics.** Fisher protected least significant difference (PLSD) tests of ANOVA was used to analyze the oviposition rate of *T. urticae* and phytohormone data. The data of gene expression were log-transformed and statistically analyzed by a one-way ANOVA. A replicated *G* test of goodness-of-fit was performed to analyze the feeding choice of *T. urticae* between treated and control leaves.

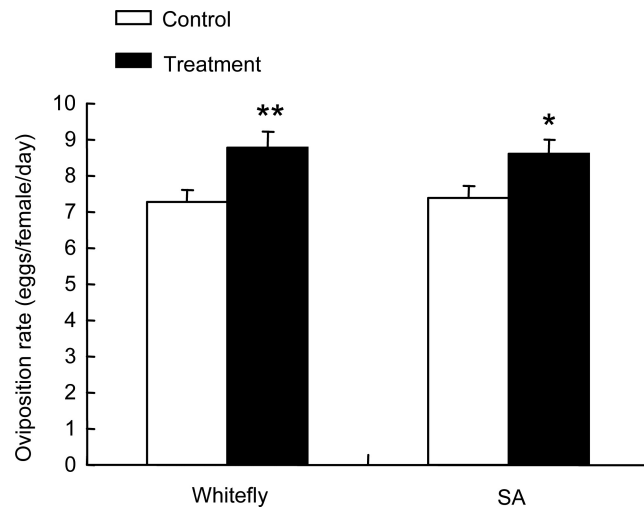
**Oviposition Rate of *T. urticae*.** The mean ( $\pm$  SE) oviposition rate of *T. urticae* on uninfested leaf discs from a *B. tabaci*-infested plant was  $8.8 \pm 0.4$  eggs/female/day, which was significantly higher than that on a leaf disc from a control plant ( $7.3 \pm 0.3$  eggs/female/day;  $P = 0.007$ ) (Fig. S1). Similarly, the mean oviposition rate of *T. urticae* on SA-treated plant leaf discs  $8.6 \pm 0.4$  eggs per female per day, which was significantly higher than that on control plant ( $7.4 \pm 0.3$  eggs per female per day;  $P = 0.015$ ; Fig. S1).

**Feeding Choice of *T. urticae*.** When *T. urticae* females were offered a choice between leaf discs from undamaged leaves from *B. tabaci*-infested and control plants, more adults chose for the leaf discs from *B. tabaci*-infested plants than for the leaf discs from control plants ( $G = 8.8$ ,  $P = 0.003$ ) (Fig. S2). Similarly, when *T. urticae* were offered a choice between discs from SA-treated plants and control leaf discs, more adults were found on discs from SA-treated plants than on control leaf discs ( $G = 9.48$ ,  $P = 0.002$ ) (Fig. S2). Which cues mediate the discrimination by the spider mites remains to be unraveled. They can be either plant volatiles or nonvolatile cues within the leaf or on its surface. For example, it has been previously reported that whiteflies (*Trialeurodes vaporariorum*) induce odors in bean plants (*Phaseolus vulgaris*) (1). For the present context it suffices that indeed spider mites prefer to feed on leaves from plants that are infested with *B. tabaci* or treated with SA and which represent a better resource to the spider mites in terms of increased reproduction and reduced indirect defense than leaves from untreated control plants.

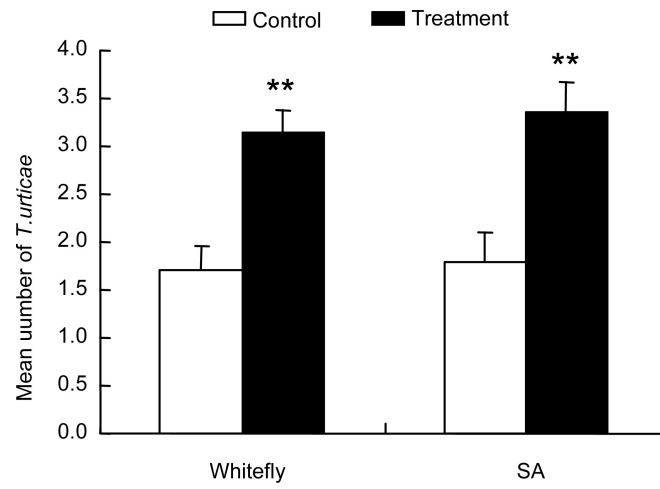
**Time Course of SA Accumulation.** The amount of SA was significantly reduced in leaves simultaneously infested with 10 *T. urticae* and 50 *B. tabaci* compared to leaves infested with 10 *T. urticae* at 12 h after infestation ( $F_{1,8} = 34.4$ ,  $P < 0.001$ ). After 3 and 7 days since infestation the SA level increased and was similar to that in plants infested with 10 *T. urticae* only (Fig. S3).

**Time Course of *PIOS* Expression.** No induced transcripts of *PIOS* were detected either in leaves infested with 10 *T. urticae* or leaves infested with 10 *T. urticae* and 50 *B. tabaci* after 12 h of infestation (Fig. S4). After 3 days of infestation, *B. tabaci* did not affect the *PIOS* transcript levels induced by *T. urticae*; while after 7 days of infestation, *B. tabaci* caused a marginally significant reduction in the *PIOS* transcript levels induced by *T. urticae* ( $F_{1,4} = 6.5$ ,  $P = 0.06$ ; Fig. S4).

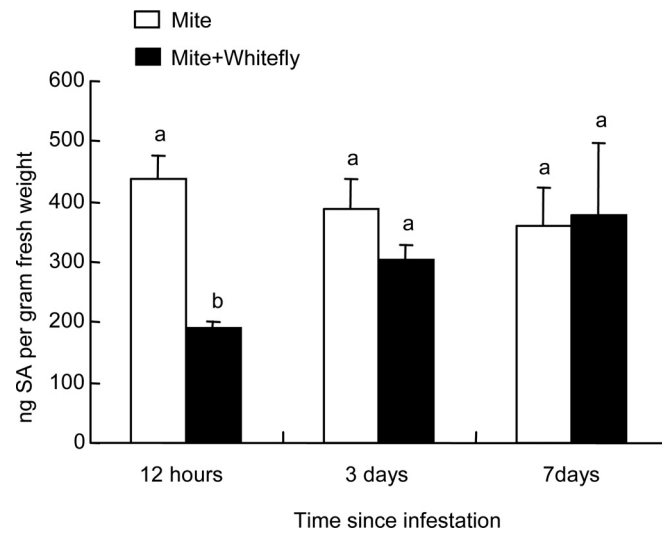
1. Birkett MA, Chamberlain K, Guerrieri E, Pickett JA, Wadhams LJ, Yasuda T (2003) Volatiles from whitefly-infested plants elicit a host-locating response in the parasitoid, *Encarsia formosa*. *J Chem Ecol* 29:1589–1600.



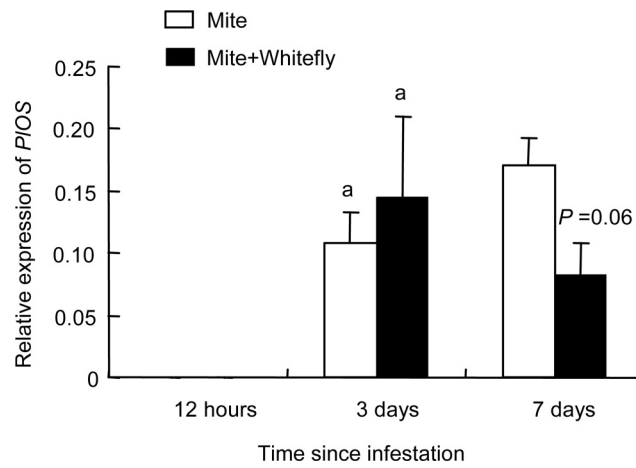
**Fig. S1.** Mean oviposition rate ( $\pm$ SE) of *T. urticae* on leaf discs from undamaged plants (Control) and on leaf discs from *B. tabaci*-infested plants or on leaf discs from SA-treated plants versus undamaged plants (Treatment) ( $n = 10$ ). Asterisks represent significant differences from control plants as determined by Fisher protected least significant difference (PLSD) tests of ANOVA (\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ).



**Fig. S2.** Mean ( $\pm$ SE) number of *T. urticae* (out of six individuals) found on leaf discs from undamaged (Control) and *B. tabaci*-infested or SA-treated (Treatment) plants ( $n = 20$ ). Asterisks represent significant differences from control plants as determined by replicated *G* test of goodness-of-fit (\*\*,  $P < 0.01$ ).



**Fig. S3.** Amount of SA in leaves infested with 10 *T. urticae* only and in leaves simultaneously infested with 10 *T. urticae* and 50 *B. tabaci* at different time points. Values are the mean ( $\pm$ SE) of 3–5 biological replicates. Different letters above bars indicate significant differences in the transcript levels between treatments (Fisher's PLSD test of ANOVA,  $P < 0.05$ ).



**Fig. S4.** qRT-PCR data for *PIOS* gene expression. Transcript levels of *PIOS* in leaves infested with 10 *T. urticae* only and leaves simultaneously infested with 10 *T. urticae* and 50 *B. tabaci* at different time points. *PIOS* transcript levels have been normalized to the amount of *PIACT1* transcripts in each sample. Values are the mean ( $\pm$ SE) of three biological replicates. Different letters above bars indicate significant differences in the transcript levels between treatments (Fisher's PLSD test of ANOVA,  $P < 0.05$ ).