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Reduction in the Fitness of *Bemisia tabaci* Fed on Three Previously Infested Tomato Genotypes Differing in the Jasmonic Acid Pathway

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ABSTRACT The effect of previous infestation (preconditioning) by the whitefly *Bemisia tabaci* (Gennadius) biotype B on the population fitness of subsequent infestations that fed on three isogenic tomato genotypes (wild-type [Wt], a jasmonic acid [JA] defense-enhanced genotype [*35S*], and a JA-deficient genotype [*spr2*]) was examined. We tested the hypotheses that whiteflies fed on preconditioned tomatoes (*Solanum lycopersicum* L.) would have reduced fitness and that the effect would be mediated via the JA-dependent systemic plant defense pathway. Preconditioning by the whitefly resulted in decreased levels of soluble sugars and free amino acids and increased salicylic acid (SA), total phenolics, and condensed tannins for all three genotypes. The durations of the larval and pupal stages were prolonged in whiteflies fed on the preconditioned plants compared with those that fed on control plants. Furthermore, preconditioning resulted in reduced fecundity and intrinsic rate of increase (r_m) of the whiteflies that subsequently fed on the three tomato genotypes. Whiteflies were more likely to feed and deposit eggs on control plants than on preconditioned plants. Our results indicate that preconditioning induced decreases in leaf nutrients and increased induction of an SA based defense that degraded the quality of the substrate as evidenced by an increased developmental time and reduced fecundity of whiteflies that subsequently fed on them.

KEY WORDS *Bemisia tabaci*, jasmonic acid, salicylic acid, tomato, whitefly preconditioning

Plant responses to herbivore damage may consist of physiological or morphological changes that can affect the performance and preference of subsequent herbivores (Karban and Baldwin 1997, Kessler and Baldwin 2001, Rodriguez-Saona et al. 2005). Temporal and spatial interactions of herbivorous insects with other intra- or interspecific insects are mediated by changes in the target plant quality (Ohgushi 2005). Intraspecific insect interactions were considered more important in determining population densities and structuring communities (Strong et al. 1984). Notably, phloem-sucking insects that feed exclusively on the sieve elements of plants were sensitive to nutritional content and changes in the resistant compounds in the phloem sap, which may be induced by other species of insects (Denno and Roderick 1992, Denno et al. 1995, Wu and Baldwin 2009).

Insect feeding could influence a plant's nutrient availability by altering the source-sink relationships and thus impact the performance of later-arriving species (Larson and Whitham 1991, Petersen and Sandström 2001). For example, a whitefly infestation reduced the level of nitrogen and total carbohydrates and increased the concentration of several phenolic compounds in plant tissues (Mayer et al. 2002, Chen et al. 2004b, Murugan and Dhandapani 2007). The amino-acid and carbohydrate levels positively correlated with the density and development of whiteflies (Bi et al. 2003). In contrast, the secondary metabolites (i.e., jasmonic acid [JA], salicylic acid [SA], phenolics, and terpenes), deterred the feeding and oviposition of piercing–sucking insects and decreased their population fitness (Mansour et al. 1997, Jormalainen et al. 2001, Pegadaraju et al. 2005, Sanchez-Hernandez et al. 2006, Zarate et al. 2007). Because of alterations in the plant biochemistry after an insect infestation, the settling, feeding, growth, development, and fecundity of later-arriving herbivores may be affected (Inbar et al. 1999, Bi et al. 2003, Ohgushi 2005).

Plant defenses are regarded as an indispensable factor influencing insect–plant interactions (Mauricio et al. 1997, Pegadaraju et al. 2005, Kusnierczyk et al. 2008). In general, leaf-chewing insects largely stimulated the JA-dependent defenses, whereas pierce-sucking insects triggered SA-dependent defenses (Heidel and Baldwin 2004, Zarate et al. 2007). A whitefly infestation upregulated the expression of the downstream defense genes (i.e., the pathogenesis-related proteins), of the SA signaling pathway (Walling 2000), which equipped the plant to resist aphids (Cooper et al. 2004, Pegadaraju et al. 2005, Li et al. 2006). Com-

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pared with the SA-mediated local, instant, and weak resistance against herbivorous insects, the JA pathway induced a systemic and strong resistance against herbivorous insects (Truman et al. 2010). Upregulated JA-dependent defenses also slow whitefly nymph development and aphid population expansion (Zarate et al. 2007, Walling 2008). Thus, the JA-dependent pathway of plants may be involved in mechanisms that mediate the temporal and spatial interactions of intra- and interspecific insects (Li et al. 2003). Most researchers have assumed that the induced defenses of plants against whiteflies depend on the SA signaling pathway (Kempema et al. 2007), and it remains unclear whether JA-enhanced genotypes or deletion mutants would affect the performance of whiteflies.

Bemisia tabaci (Gennadius) (Homoptera: Aleyrodidae) (Gennadius 1889) is one of the most noxious insect pests in field and greenhouse crops worldwide (Bird and Krüger 2006). Biotype B is now the predominant or only biotype of *B. tabaci* in many regions of China (Jiu et al. 2007). The effects of preconditioning on three tomato (*Solanum lycopersicum* L.) genotypes, including the wild-type, a JA defense-enhanced genotype (*35S*) and a JA mutant (*spr2*), were examined with respect to the phloem-feeder *B. tabaci* biotype B. Our previous research has indicated that an artificial enhancement of the JA pathway increased the resistance against the tobacco hornworm *Manduca sexta* (L.) and the subterranean nematode *Meloidogyne incognita* (Kofoid and White 1919) Chitwood 1949 (Li et al. 2003, Sun et al. 2011). Testing of these JA-enhanced genotypes for effects on temporal and spatial interactions with piercing and sucking insects have not been conducted and thus we tested whether whitefly preconditioning would decrease the nutrient content and increase secondary metabolites in the plants thereby reducing the population fitness of subsequent whiteflies, and whether a JA-dependent systemic defense may also affect these interactions. Our specific objectives were to determine the following: 1) the effects induced by whitefly preconditioning on the fitness of subsequent feeding by *B. tabaci*, 2) the effects induced by whitefly preconditioning on the feeding and oviposition preferences of subsequent infestations of *B. tabaci*, and 3) whether such effects are mediated via the JA-dependent systemic defensive pathway.

Materials and Methods

Open-Topped Plexiglas Cylinders. The experiments were conducted in a field laboratory in Xiaotangshan County, Beijing, China (40° 11′ N, 116° 24′ E) with eight open-topped Plexiglas cylinders (2.2 m in height and 2 m in diameter) used to house the potted tomato plants; four cylinders were used for the preconditioning treatments, and the remaining four were used for the control treatments.

Host Plants. Three tomato genotypes were selected for the current study: wild-type (Wt) tomato plants (*L. esculentum* 'Castlemart'), jasmonate-deficient *spr2* mutant tomato plants (*spr2*), and *35S::prosystemin* transgenic tomato plants (*35S*). These plants were provided by Professor C. Li of the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences. *L. esculentum* Castlemart was the Wt parent for the *spr2* mutant and the *35S* transgenic plants. The *35S::prosystemin* (*35S*) JA-biosynthesis mutant transgenic plants overexpress prosystemin, which constitutively activates the systemic defenses in unwounded plants and results in a stronger and more rapidly induced resistance. In contrast, the suppressor of prosystemin-mediated responses2 (*spr2*) mutant exhibits reduced levels of chloroplast *w3* fatty acid desaturase, which impairs the synthesis of JA (McGurl et al. 1992, 1994). After being grown in sterilized soil for 2 wk, the tomato seedlings were transplanted individually into plastic pots (14 cm in diameter, 12 cm in height) containing sterilized loamy field soil. Plants that were ≈40 d old with heights of ≈20–30 cm were moved to the open cylinders on 27 July 2009. Each open cylinder contained 18 plants (six individuals from each tomato genotype × three genotypes).

Preconditioning Treatments. Specimens of *B. tabaci* biotype B were collected on 5 April 2009, from cabbage (*Brassica oleracea* L.) plants at the Agriculture and Forest Academy of Beijing, China. The offspring of these whiteflies were reared on tomatoes. For the preconditioning treatments, three secondary branches of each tomato plant were used, three terminal leaves of each secondary branch were encased in a mesh gauze bag and infested with 90 male whiteflies (to avoid the production of offspring) at various times during the 28 July to 4 October 2009 period; subsequent whitefly performance was assessed using a lateral leaf from the same secondary branch. The 90 male whiteflies, used for the preconditioning treatments, were placed on tomato plants 1 d earlier than subsequent challenge infestations (assessments of whitefly performance) and maintained by replacement every 3 d until assessments of the challenge infestations were complete. Subsequent whitefly performance was established on 29 July 2009. This work was done in the open-topped Plexiglas cylinders (Supp Fig. 1).

The preconditioning treatment methods for plants used for chemical analyses were the same as those in the above preconditioning treatments. The tomato leaves (lateral leaves from the same secondary branch with the preconditioning treatments) exposed to subsequent infestations were collected at 1- and 3-wk intervals after infestation, and frozen at −20 and −78°C for later chemical analysis.

Developmental Time, Fecundity, and Adult Longevity of Subsequent *B. tabaci* Infestation. Tomato plants that were ≈40 d old with heights of ≈20–30 cm were selected from stock populations of these cultivars and moved to each open cylinder, and three terminal leaves of each plant were inoculated with 10 pairs of whitefly adults on each leaf in a clip cage (diameter: height is 3.5: 1.5 cm) on 29 July 2009. Therefore, a total of 12 tomato leaves were inoculated with whitefly adults for each cultivar in each infestation treatment. The adults were removed after 24 h,

and 30 eggs were left on each leaf. The developmental time for *B. tabaci* was recorded daily by using a microscope until adult eclosion. After adult eclosion, pairs of newly eclosed adults from each treatment were transferred to another leaf of the same tomato plant in the same open canopy using a clip cage. If a male died, another healthy male from the same treatment immediately was added. The adult longevity and fecundity of each individual whitefly was recorded daily. The experiment was done from 29 July to 4 October 2009.

Feeding and Oviposition Preferences of *B. tabaci*. One control plant and one preconditioned plant of each tomato genotype were placed into the same cage (dimensions of 60 by 60 by 60 cm) on 7 August 2009 in the open cylinders. They were control Wt plant/preconditioned Wt plant, control *35S* plant/preconditioned *35S* plant, and control *spr2* plant/preconditioned *spr2* plant, respectively. The experiment was conducted with 15 replications. For the preconditioning treatment, a cohort of 90 male whiteflies was established and replaced every 3 d on the lateral three leaves to provide a continuous infestation for 3 wk in the open cylinders. After 3 wk, 50 pairs of adult whiteflies were put into each cage, and 3 d later, the feeding and oviposition preference of each whitefly was recorded.

The other experiment is a group of the same treatment of different tomato plants, consisting of 15 control treatment cages and 15 preconditioned treatment cages, with dimensions of 60 by 60 by 60 cm, on 12 August 2009 in the open cylinders. Each of 15 cages contained three control plants (one wt, one *35S* and one *spr2* plant). Each of the other 15 cages contained three preconditioned treatment plants (one preconditioned wt, one preconditioned *35S*, and one preconditioned *spr2* plant). The preconditioned treatment was the same with the above experiment. After 3 wk, 50 pairs of adult whiteflies were put into each cage, and 3 d later, the feeding and oviposition preference of each whitefly was recorded.

Analysis of Free Amino Acids and Soluble Sugars. The free amino acids were extracted according to the procedure of Chen et al. (2004a). A leaf tissue sample of 0.5 g was homogenized on ice, and 10 ml of ethanol (70%, V/V) was added. The samples were centrifuged at 1,000 g for 5 min after boiling and cooling. The supernatant was transferred to graduated vessels. Five milliliters of phosphate buffer (pH 8.0) and ninhydrin-ethylene glycol (3%, W/V) were added. This mixture was heated, and 70% ethanol was added to a total volume of 50 ml. This solution was agitated, and the free amino acids were measured subsequently at 570 nm with a spectrophotometer (DU 800, Beckman Coulter, Brea, CA) by using ninhydrin reagent (Sigma-Aldrich, St. Louis, MO). For the calculation of the free amino acids concentrations, a standard curve was prepared using glycine.

The soluble sugars were extracted according to the procedure of Irigoyen et al. (1992) with a slight modification. The soluble sugars were extracted from 0.5 g of lyophilized leaves by using 15 ml of distilled water. The samples were centrifuged at 1,000 g for 5 min after boiling and cooling. The supernatant was transferred into a 100-ml volumetric flask and brought to volume with distilled water. The soluble sugars were measured at 620 nm by using a spectrophotometer (DU 800, Beckman Coulter) and anthrone reagent. The concentration of soluble sugars was estimated using the anthrone method with glucose as the standard (Irigoyen et al. 1992).

SA Measurements. The SA was extracted and quantified as described by Ren et al. (2010) with slight modifications. A sample of 0.5 g of frozen leaf tissue was homogenized on ice, extracted with 3 ml of 90% methanol and centrifuged at 8,000 g for 20 min at 4°C. The supernatant was extracted again using 2 ml of pure methanol and centrifuged. The merged supernatant was dried at 60°C in a kettle in a water bath, and 1.5 ml of 5% trichloroacetic acid was added. This mixture was centrifuged at 7,500 g for 15 min and was extracted three times with a mixture comprised of equal volumes of ethyl acetate and cyclohexane. The organic phase containing the free SA was subsequently dried in a speed vacuum. After the evaporation of the solvent in the collected sample, 500 μl of acetonitrile was added to the residue. The resulting solution was filtered through a 0.45 μm filter, and the filtered liquor was analyzed using high performance liquid chromatography (HPLC).

The fractions generated during the HPLC analysis of the endogenous free SA were collected by injecting 20 μl of the sample into a C_{18} reversed-phase column (5 μm, 250 mm by 4.6 mm). The column was maintained at 40°C with a gradient of 0.8 ml/min flow programmed as follows: 0/100 for 5 min, 60/40 for 30 min, 80/20 for 35 min, to 0/100 with a 40 min hold in acetonitrile/H_2O containing 0.5% acetic acid. The UV absorption was monitored at 295 nm, and the data were analyzed using ChromQuest. The HPLC system consisted of a G1313A autosampler, a G1313A Quarpump, a G1315B DAD Detector, a G1316A Column temperature box and a G1379A DeGAS Series (Agilent Technologies Inc., Santa Clara, CA). SA was measured by comparing the retention time and the scan spectra library of the standard for SA. The retention time of SA was identified using a data system, and the quantitative analysis of SA was completed by plotting the results against the standard curve.

Endogenous JA Measurements. According to the procedures described by Ren et al. (2010), 0.5 g of fresh leaf tissue was ground into a fine powder on ice, mixed with 4 ml of 80% methanol (V/V), and kept at −20°C for 12 h. This mixture then was added to 6 μl of [9, 10]-dihydro-JA to be used as an internal standard. The total extracted preparation was centrifuged at 8,000 g for 20 min.

The supernatant was condensed to an aqueous phase after the methanol was vaporized. The aqueous sample was frozen at −20°C and later thawed, and this freezing process was repeated three times. After the third thawing, the extract was centrifuged at 3,000 g for 20 min. The pH of the supernatant was adjusted to

2.5–3.0 using HCl (≈2 mol/liter). The supernatant was extracted with an equal volume of ethylacetate and was dried. The dried extract was resuspended in 0.1-M acetic acid and was loaded onto a C_{18} column (Waters Company, Milford, MA). After loading, the C_{18} column was sequentially eluted with a series of solvent mixtures, collecting 5 ml of the solvent mixture each time. The series consisted of acetic acid/methanol (V/V) at 83/17, 60/40, and 40/60. The last 4 ml that were eluted in 40% methanol and the first 3 ml that was eluted in 60% methanol was collected. After the evaporation of the solvent and the esterification of the residue with excess diazomethane, the elution sample volume was adjusted to 50 μl with acetic acid and analyzed using gas chromatography-mass spectroscopy (GC-MS).

The GC-MS analysis of the extracts of the endogenous JA was conducted using a DB-5-MS column (30 m by 0.32 mm by 0.25 μm; J&W Scientific, Agilent Technologies, Santa Clara, CA). Helium was used as the carrier gas with a constant flow rate of 0.8 ml/min. For each extract sample, 1 μl was injected in splitless mode. The injector temperature was 280°C, the GC-MS transfer line temperature was 250°C and the source temperature was 200°C. The emission current was 150 μA, the detector voltage was 500 V, the ionization potential was 70 eV, the scan speed was 0.4 s and the scan range was 29–450 m/z. The GC was programmed for an initial oven temperature of 50°C, a temperature increase at the rate of 20°C min^{-1} up to 180°C, a 4 min hold, an increase of 10°C min^{-1} up to 220°C and a final 15 min hold.

The endogenous JA and its internal standards (Dihydro-JA) were analyzed using full GC/MS scans. The retention times were identified using Xcalibur 1.2 and the NIST 2003 mass library and retention time. The endogenous JA was measured using GC-MS selected ion monitoring. The characteristic ions of JA (m/z) and the internal standard [9, 10]-dihydro- JA (m/z) were 151/224 and 153/226, respectively.

Analysis of Total Phenolics and Condensed Tannins. The total phenolics were extracted according to the procedure described by Kujala et al. (2000), A 0.1 g sample of dried leaves was dissolved in 10 ml of methanol/ water mixture (50:50, vol:vol). The sample solution (1 ml) was mixed with 1 ml volume of Folin-Ciocalteu reagent. The mixture was allowed to stand for 5 min, added to 2 ml of 20% Na_2CO_3 and subsequently left for 10 min. The mixture was centrifuged for 8 min (12,000 g). The supernatant was measured at 730 nm using a spectrophotometer (DU 800, Beckman Coulter). To calculate the concentrations of the total phenolics, a standard curve was prepared using gallic acid.

The condensed tannins were extracted according to the procedure described by Terrill et al. (1992) with slight modifications. A 0.1 g sample of dried leaves was shaken with 2.5 ml methanol-HCI (10:1, V/V) and left for 24 h at room temperature. An aliquot of 1.5 ml of the supernatant from this extraction was transferred into a test tube. A 3-ml volume of vanillin and methanol reagent (4% W/V in methanol) and 1.5 ml of HCl were added to the test tube, which was wrapped in aluminum foil to protect the sample from light. The mixture was incubated in a water bath for 20 min at 20°C. The condensed tannins were measured at 510 nm by using a spectrophotometer (DU 800, Beckman Coulter). For calculating the concentrations of the condensed tannins, a standard curve was prepared using catechin.

Population Parameter Estimation. The intrinsic rate of increase (r_m), net reproduction (R_0), and mean generation time (T) were analyzed based on the age-stage, two-sex life table model developed by Chi and Liu (1985) and Chi (1988). The jackknife method was used to estimate the means and standard errors for the population parameters (Sokal and Rohlf 1995). The TWOSEX-MSChart computer program (Chi 2004) was developed for the data analysis and jackknife estimation in Visual Basic for the Windows operating system (Yin et al. 2009).

Statistical Analyses. A split-plot design, with the whitefly preconditioning being the main factor and the tomato genotype serving as a subfactor, was used for initial analyses. Population traits, including the total duration of the larval and pupal stages, fecundity, and intrinsic rate of increase (r_m) in the whiteflies were analyzed using a split-plot analysis of variance (ANOVA) (SAS 6.12, SAS Institute Inc., 1996). For the chemical composition of the tomatoes, whitefly preconditioning was the main factor and the tomato genotype and time period served as sub-factors that were analyzed using a split-plot ANOVA (SAS 6.12, SAS Institute, 1996). In addition, Pearson's correlations were calculated to analyze the relationships among the developmental time, fecundity and r_m for *B. tabaci* and the SA, soluble sugars, free amino acids, total phenolics, and condensed tannins in the tomatoes reared either under preconditioning or without preconditioning of whiteflies. χ^2 tests were used to analyze the adult whitefly feeding and oviposition preference.

Results

Developmental Time in *B. tabaci*. The whitefly preconditioning increased the total duration of the larval and pupal stages of the subsequent feeding whiteflies by 7.16% for the Wt genotype ($F = 17.424$; df = 1, 142; $P < 0.001$); by 5.48% for the *35S* genotype ($F = 22.211$; df = 1, 142; $P < 0.001$); and by 5.98% for the *spr2* genotype ($F = 19.600$; df = 1, 142; $P < 0.001$) (Table 1, Fig. 1a).

Significant differences were observed in the total duration of the larval and pupal stages ($F = 6.072$; df = 2, 213; $P = 0.003$) for whiteflies feeding on different genotypes (Table 1, Fig. 1a). Tomato genotype influenced the total duration of the larval and pupal stages ($F = 3.813$; df = 2, 213; $P = 0.024$) on plants without whitefly preconditioning (Table 1, Fig. 1a). Regardless of the whitefly preconditioning, the total duration of the larval and pupal stages of the subsequent feeding whiteflies on the *35S* plants was longer than the same stage on the *spr2* plants (Fig. 1a).

Table 1. *P* values for the effects of whitefly preconditioning and tomato genotypes on the population parameters of subsequently feeding *B. tabaci*, as determined using split-plot ANOVAS

Treatments	Duration of the larval and pupal stages	Fecundity	r_m[a]
Preconditioning	0.003	0.006	0.000
Genotype	0.000	0.606	0.002
Preconditioning × genotype	0.634	0.891	0.317

[a] The intrinsic rate of increase.

Fecundity of *B. tabaci*. The whitefly preconditioning decreased the fecundity of the subsequent feeding whiteflies by 28.93% for the Wt genotype ($F = 7.943$; df = 1, 44; $P = 0.007$); by 22.76% for the *35S* genotype ($F = 9.362$; df = 1, 44; $P = 0.004$); and by 19.33% for the *spr2* genotype ($F = 8.348$; df = 1, 44; $P = 0.006$) (Table 1, Fig. 1b). The different tomato genotypes did not significantly affect the whitefly fecundity either after preconditioning of the plants ($F = 0.804$; df = 2, 66; $P = 0.425$) or without preconditioning ($F = 0.108$; df = 2, 66; $P = 0.898$) (Table 1, Fig. 1b).

Intrinsic Rate of Increase (r_m) in *B. tabaci*. The whitefly preconditioning decreased the r_m of the subsequent feeding whiteflies by 7.82% for the Wt genotype ($F = 6.998$; df = 1, 6; $P = 0.038$), by 6.13% for the *35S* genotype ($F = 6.742$; df = 1, 6; $P = 0.041$); and by 12.16% for the *spr2* genotype ($F = 16.380$; df = 1, 6; $P = 0.007$) (Table 1, Fig. 1c). The different tomato genotypes significantly influenced the r_m with ($F = 6.922$; df = 2, 9; $P = 0.015$) and without ($F = 6.819$; df = 2, 9; $P = 0.016$) whitefly preconditioning of the plants (Table 1, Fig. 1c). Regardless of the whitefly preconditioning, the r_m of the subsequent feeding whiteflies on the *35S* plants was lower than those on the *spr2* plants (Fig. 1c).

Feeding and Oviposition Preferences of *B. tabaci*. Plants with no preconditioning were preferred by adult whiteflies for feeding (Wt: $\chi^2 = 8.247$, $P = 0.004$; *35S:* $\chi^2 = 15.311$, $P < 0.001$; *spr2:* $\chi^2 = 14.442$, $P < 0.001$) and oviposition (Wt: $\chi^2 = 128.971$, $P < 0.001$; *35S:* $\chi^2 = 77.118$, $P < 0.001$; *spr2:* $\chi^2 = 161.263$, $P < 0.001$) (Fig. 2a and b).

The Wt plants were preferred by adult whiteflies for feeding ($\chi^2 = 13.072$, $P = 0.001$) and oviposition ($\chi^2 = 439.292$, $P < 0.001$) without whitefly preconditioning. Regardless of the whitefly preconditioning, the *35S* plants were not preferred by adult whiteflies for feeding and oviposition (Fig. 3a and b).

Soluble Sugar and Free Amino Acid Contents in the Tomatoes. The whitefly preconditioning decreased the soluble sugar content in the Wt ($F = 10.860$; df = 3, 8; $P = 0.003$); *35S* ($F = 12.765$; df = 3, 8; $P = 0.002$); and *spr2* ($F = 11.653$; df = 3, 8; $P = 0.003$) tomatoes by 24.07, 23.77, and 25% after 1 wk and by 29.29, 29.20, and 29.27% after 3 wk, respectively (Table 2, Fig. 4a). The soluble sugar content in the leaves of the *spr2* plant was higher than the content of the *35S* plant after the whitefly preconditioning for 1 and 3 wk (Fig. 4a). The soluble sugars were also higher in the *spr2* plant than the content of the *35S* plant at 1 wk without whitefly preconditioning (Fig. 4a).

The whitefly preconditioning decreased the free amino acid content of the Wt ($F = 10.155$; df = 3, 8; $P = 0.004$); *35S* ($F = 11.304$; df = 3, 8; $P = 0.003$); and *spr2* ($F = 13.489$; df = 3, 8; $P = 0.002$) tomatoes by

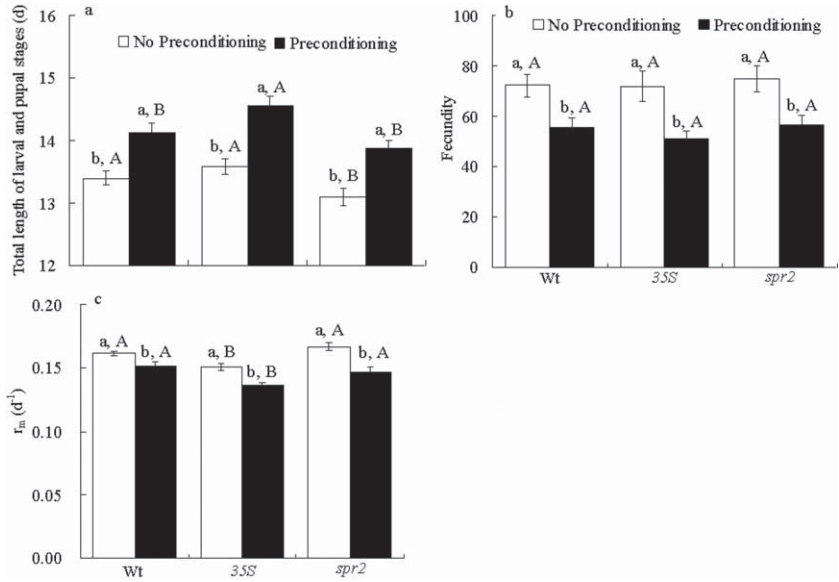

Fig. 1. Developmental time of the duration of the larval and pupal stages (a), fecundity (b) and r_m (c) for whiteflies reared on the three tomato genotypes (Wt, *35S*, *spr2*) with or without preconditioning by whiteflies. Each value represents the average ± SE. Different lowercase letters indicate significant differences between the infestation treatments in a specific tomato cultivar (least significant difference (LSD) test: $P < 0.05$); different uppercase letters indicate significant differences between the tomato cultivars at an infestation level (LSD test: $P < 0.05$).

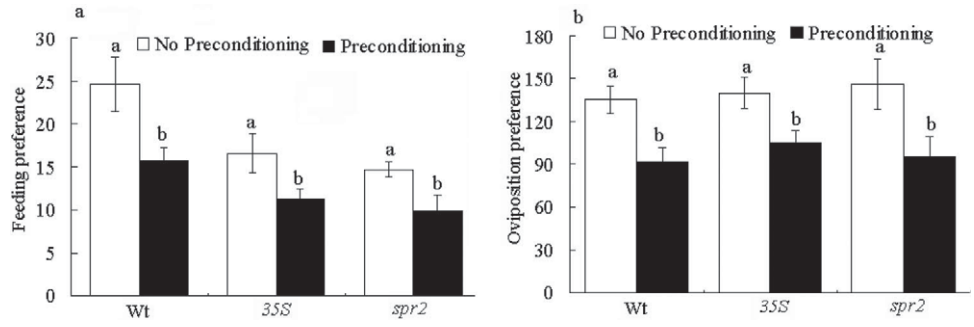

Fig. 2. Effect of whitefly preconditioning on the feeding (a) and oviposition (b) preferences of subsequent feeding whiteflies fed on three tomato genotypes after three weeks. Each value represents the average (±SE) of 15 replicates. Different lowercase letters indicate significant differences between the infestation treatments for a specific tomato cultivar (χ^2 test: $P < 0.05$).

25.80, 24.62, and 21.01% after 1 wk and by 29.56, 33.74, and 35.62% after 3 wk, respectively (Table 2, Fig. 4b). The free amino acid contents were higher in the *spr2* plant than that in the *35S* plant at 3 wk without whitefly preconditioning (Fig. 4b).

Free SA and JA Content in the Tomatoes. The whitefly preconditioning increased the free SA content in the Wt ($F = 26.879$; df = 3, 8; $P < 0.001$); *35S* ($F = 15.840$; df = 3, 8; $P = 0.001$); and *spr2* ($F = 30.179$; df = 3, 8; $P < 0.001$) tomatoes by 94.60, 48.19, and 40.47%, respectively, after 1 wk and 1.25-, 1.05-fold, and 86.09%, respectively, after 3 wk (Table 2, Fig. 5a). Regardless of whitefly preconditioning, the different genotypes had no influence on the free SA content in the tomatoes (Table 2, Fig. 5a). The free SA content of the three tomato genotypes significantly increased between 1 and 3 wk after whitefly preconditioning (Fig. 5a).

The whitefly preconditioning and time period did not influence the JA content in the tomatoes (Table 2, Fig. 5b). Regardless of whitefly preconditioning, the JA content in the tomatoes of the *35S* plants was higher than that of the *spr2* plants after 1 and 3 wk (Fig. 5b).

Total Phenolics and Condensed Tannins Content in the Tomatoes. The whitefly preconditioning increased the total phenolics content of the Wt ($F = 50.499$; df = 3, 8; $P < 0.001$); *35S* ($F = 17.233$; df = 3, 8; $P < 0.001$); and *spr2* ($F = 17.450$; df = 3, 8; $P < 0.001$) tomatoes 1.46-, 1.29-, and 1.53-fold after 1 wk and 1.32-, 1.08-, and 1.38-fold after 3 wk, respectively (Table 2, Fig. 6a). The whitefly preconditioning increased the condensed tannins content of the Wt ($F = 13.207$; df = 3, 8; $P = 0.002$); *35S* ($F = 20.491$; df = 3, 8; $P < 0.001$); and *spr2* ($F = 19.772$; df = 3, 8; $P < 0.001$) tomatoes by 78.26, 75.65, and 77.78%, respectively, after 1 wk and by 76.58, 55.28, and 60.56, respectively, after 3 wk (Fig. 6b).

Regardless of whitefly preconditioning, the different genotypes and time period had no influence on the total phenolics and condensed tannins content in the tomatoes (Table 2, Fig. 6a and b).

Pearson Correlations. The rate of developmental time, the fecundity, and r_m of the whiteflies positively correlated with the tomato soluble sugar and free amino acid contents after 1 and 3 wk. The rate of developmental time, the fecundity, and r_m of the whiteflies negatively correlated with the tomato free SA, total phenolics, and condensed tannins contents after 1 and 3 wk. The rate of developmental time of the whiteflies negatively correlated with the tomato JA content after 1 and 3 wk (Table 3).

Discussion

Interspecific insect interactions were able to influence the species richness and diversity in ecosystems

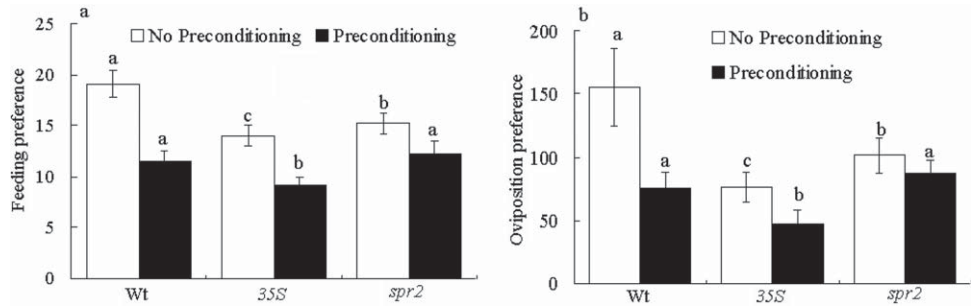

Fig. 3. Effect of the tomato genotypes on the feeding (a) and oviposition (b) preferences of subsequent feeding whiteflies after 3 wk with or without whitefly preconditioning. Each value represents the average (±SE) of 15 replicates. Different lowercase letters indicate significant differences between the tomato cultivars with and without preconditioning (χ^2 test: $P < 0.05$).

Table 2. ***P*** **values for the effects of the whitefly preconditioning, tomato genotypes, and time period on the physical indices of the tomato plants, as determined using split-plot ANOVAS**

Treatments	Soluble sugars	Free amino acids	Free SA[a]	JA[b]	Total phenolics	Condensed tannins
Preconditioning	0.000	0.000	0.001	0.065	0.000	0.000
Genotype	0.000	0.023	0.153	0.000	0.051	0.054
Time period	0.005	0.120	0.000	0.464	0.783	0.162
Preconditioning × genotype	0.696	0.803	0.132	0.222	0.969	0.861
Preconditioning × time period	0.618	0.175	0.000	0.039	0.631	0.540
Genotype × time period	0.989	0.609	0.487	0.783	0.829	0.834
Preconditioning × genotype × time period	0.999	0.500	0.936	0.320	0.983	0.881

[a] Free salicylic acid.
[b] Jasmonic acid.

(Ohgushi 2005). In terms of temporal and spatial interactions, intraspecific insect interactions affect the population fitness of each other (Hunter and Price 1992, Messina et al. 2002). However, the outcomes of insect interactions mediated by plants are often inconsistent and depend on the insect and plant species being investigated (Walling 2000, Ohgushi 2005). Previous infestations of *Macrosiphum euphorbiae* have been shown to reduce the density of *B. tabaci* (Nombela et al. 2009), whereas a preconditioning of the red alder by western tent caterpillar increased the pupal size and pupation rate of the fall webworm *Hyphantria cunea* (Williams and Myers 1984). Likewise, the current study found that whitefly preconditioning prolonged the developmental time and reduced the fecundity and r_m of the subsequent feeding whiteflies. Changes in the plant primary and secondary metabolites induced by insect infestation altered the nutritional quality and palatability of the plant, which may affect the later performance of herbivorous insects infesting the plant (Inbar et al. 1995, Rodriguez-Saona et al. 2005).

Plant nutritional content, particularly with respect to amino acid and carbohydrate levels, were able to affect the performance of phloem sap-feeding insects (Blackmer and Byrne 1999, Kainulainen et al. 2000). Phloem-feeding insects are intriguing because of their feeding mechanisms, which cause little damage to the plant tissue as they establish direct access to the amino acids and carbohydrates via the vascular tissue (Zarate et al. 2007, Wu and Baldwin 2009). The nitrogen present in phloem sap as free amino acids is regarded as a key nutritional factor for the growth, reproduction, and survival of phloem-sucking insects (Weibull 1987, Sandström and Pettersson 1994, Bi et al. 2003). Positive correlations between amino acids and the population fitness of whiteflies and aphids were established in cotton and pea plants (Blackmer and Byrne 1999, Bi et al. 2001). Furthermore, soluble sugars also affect the development, fecundity, and feeding preference of phloem-feeding insects (Simpson et al. 1995, Joern and Behmer 1997, Messina et al. 2002). Our data showed that whitefly preconditioning reduced the levels of the free amino acids and soluble sugars of tomato plants, and the subsequent feeding whiteflies are more likely to prefer feeding and ovipositing on control plants than on preconditioned plants. Moreover, in terms of developmental time and fecundity, there was a positive correlation between the population fitness of *B. tabaci* and the nutritional content (i.e., soluble sugars and free amino acids) of wild-type tomato plants, as well as the genetically modified genotypes.

The regulation pattern of plant-induced defenses, such as the JA/SA signaling pathways, is crucial to the performance of herbivorous insects (Buell 1998, Pega-

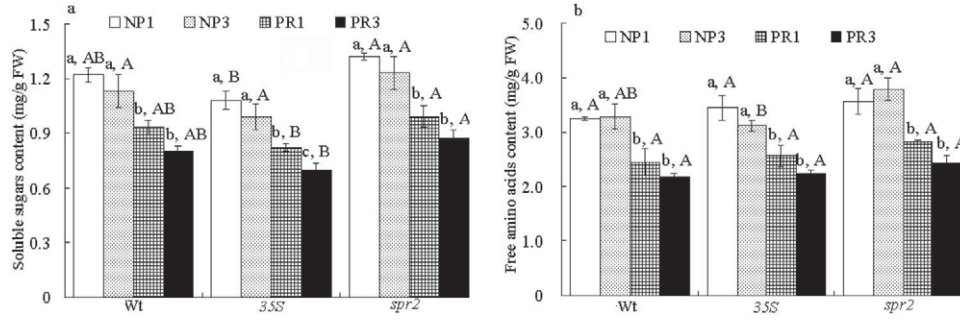

Fig. 4. The contents of soluble sugars (a) and free amino acids (b) in the three tomato genotypes (Wt, *35S*, *spr2*) grown with (PR1) or without (NP1) preconditioning by whiteflies after one week and with (PR3) or without (NP3) preconditioning by whiteflies after 3 wk. Each value represents the average (±SE) of three replicates. Different lowercase letters indicate significant differences between the infestation treatments for a specific tomato cultivar (LSD test: $P < 0.05$); different uppercase letters indicate significant differences between the tomato cultivars with and without preconditioning (LSD test: $P < 0.05$).

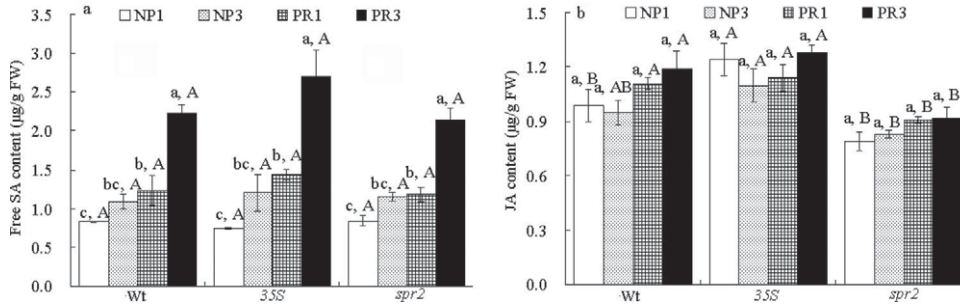

Fig. 5. The content of free SA (a) and JA (b) in the three tomato genotypes (Wt, *35S*, *spr2*) grown with (PR1) or without (NP1) preconditioning by whiteflies after 1 wk and with (PR3) or without (NP3) preconditioning by whiteflies after 3 wk. Each value represents the average (±SE) of three replicates. Different lowercase letters indicate significant differences between infestation treatments for a particular tomato cultivar (LSD test: $P < 0.05$); different uppercase letters indicate significant differences between the tomato cultivars with and without preconditioning (LSD test: $P < 0.05$).

daraju et al. 2005, Walling 2008). Although both the SA- and JA-dependent acquired resistance in plants negatively affect later-arriving phloem-feeding insects (Goggin et al. 2001, Cooper et al. 2004, Zarate et al. 2007), transcriptomic evidence has demonstrated that phloem-feeding insects tend to induce SA-dependent responses and inhibit JA signaling defenses (De Vos et al. 2005, Kempema et al. 2007, Poelman et al. 2008). The stimulated pathways of induced defenses always upregulate the synthesis of secondary metabolites, which are considered as disadvantageous to herbivorous insects because the secondary metabolites deter ingestion and cause indigestion (Walling 2000, Jormalainen et al. 2001). The whitefly preconditioning in the current study caused an increase in SA levels, total phenolics, and condensed tannins contents but did not significantly affect the JA level for any of the three tomato genotypes, suggesting that SA pathway had not effect on JA pathway in our case. In addition, the SA, total phenolics and condensed tannins contents were negatively correlated to the developmental rate and fecundity of the subsequent feeding *B. tabaci*. Besides the decreases in the nutritional content of the infested plants, the increases of secondary metabolites also had the effect of shifting the whitefly preference from preconditioned plants to control plants.

Although the SA-dependent defense pathway has been shown to be a more compatible resistance against piercing and sucking insects, the JA-regulated defenses also are involved in this interaction (Cooper et al. 2004, Li et al. 2006, Zarate et al. 2007). Our data showed that there were no differences in the SA content among three untreated tomato genotypes, whereas the *35S* tomato genotype had the highest JA content, which resulted in the lowest observed r_m and acted as a deterrent to the feeding and oviposition of whiteflies. Likewise, using the same tomato genotypes, Wei et al. (2011) showed that the extent of resistances to the *Liriomyza* leafminers correlated to the level of proteinase inhibitor (PI)-II content in these plants. Therefore, the resistance of plant to insect is most likely because of the constitutively expressed JA and PI-II content (Wei et al. 2011). Furthermore, the preconditioning treatment increased the SA but did not affect JA, thereby resulting in decreases of the population fitness of subsequent feeding whiteflies. Thus, a whitefly-induced up-regulation of the SA defense was detrimental to the performance

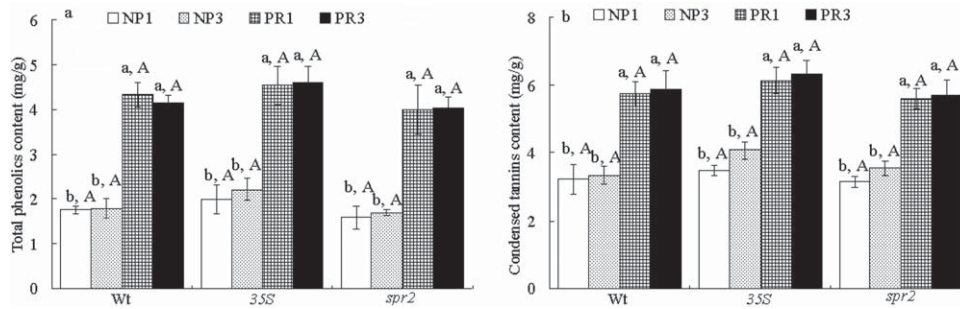

Fig. 6. The contents of total phenolics (a) and condensed tannins (b) in the three tomato genotypes (Wt, *35S*, *spr2*) grown with (PR1) or without (NP1) preconditioning by whiteflies after 1 wk and with (PR3) or without (NP3) preconditioning by whiteflies after three weeks. Each value represents the average (±SE) of three replicates. Different lowercase letters indicate significant differences between the infestation treatments for a specific tomato cultivar (LSD test: $P < 0.05$); different uppercase letters indicate significant differences between the tomato cultivars with and without preconditioning (LSD test: $P < 0.05$).

Table 3. Coefficients of Pearson correlations between the developmental time, fecundity and r_m for *B. tabaci* and the free SA, soluble sugars, free amino acids, total phenolics, and condensed tannins in the tomato plants with or without preconditioning by whiteflies

Tomato constituents	Time period	r_m[a]		Developmental time		Fecundity	
		p	r	p	r	p	r
Free SA[b]	1 wk	0.069	−0.777	0.012	0.908*	0.001	−0.969**
	3 wk	0.032	−0.850*	0.005	0.943**	0.000	−0.989**
JA[c]	1 wk	0.085	−0.752	0.024	0.871*	0.130	−0.689
	3 wk	0.081	−0.758	0.030	0.856*	0.149	−0.666
Soluble sugars	1 wk	0.004	0.949**	0.000	−0.983**	0.006	0.936**
	3 wk	0.006	0.937**	0.000	−0.979**	0.004	0.949**
Free amino acids	1 wk	0.105	0.722	0.013	−0.905*	0.003	0.953**
	3 wk	0.037	0.838*	0.006	−0.936**	0.002	0.963**
Total phenolics	1 wk	0.045	−0.821*	0.008	0.928**	0.000	−0.994**
	3 wk	0.027	−0.862*	0.005	0.939**	0.000	−0.995**
Condensed tannins	1 wk	0.04	−0.833*	0.008	0.925**	0.000	−0.995**
	3 wk	0.029	−0.856*	0.007	0.932**	0.000	−0.984**

[a] The intrinsic rate of increase.
[b] Free salicylic acid.
[c] Jasmonic acid.
* $P < 0.05$, ** $P < 0.01$.

of whiteflies. Moreover, there were no differences in the nutritional and secondary metabolite contents between the Wt plants and the *spr2* plants, but whiteflies preferred feeding and ovipositing on the Wt plants, which is consistent with the adult leafminer's preference (Wei et al. 2011). This preference may result from the volatile constituents of the different genotypes of the plants.

In summary, a previous infestation reduced the r_m of subsequent whiteflies by inducing decreases in the leaf nutrients and increasing an induced defense. Interactions between the original and subsequent whiteflies were mediated by the changes in the plant phloem sap, which did not depend on the JA pathway. Likewise, the whitefly preconditioning appeared to have significant effects on other phytophagous species that subsequently infested the previously infested plant; this result suggests that the host plant may benefit from modest levels of whitefly infestation early in the season that precondition the plant to resist subsequent infestations of numerous species of phytophagous arthropods. Whether this finding is a purposeful result of the evolution of plants in mixed-herbivore environments where bioassociations affected the natural selection or an anomalous outcome resulting from these experimental conditions warrants further study. These empirical results are linked to chemical pathways that are known to be under genetic control and thus amenable to manipulation through plant breeding, which may aid in the development of plants better suited to produce human-valued resources in the presence of a diverse pest complex.

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