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Cross-Kingdom Effects of Plant-Plant Signaling via Volatile Organic Compounds Emitted by Tomato (*Solanum lycopersicum*) Plants Infested by the Greenhouse Whitefly (*Trialeurodes vaporariorum*)

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Abstract Volatile organic compounds (VOCs) emitted from plants in response to insect infestation can function as signals for the attraction of predatory/parasitic insects and/or repulsion of herbivores. VOCs also may play a role in intra- and inter-plant communication. In this work, the kinetics and composition of VOC emissions produced by tomato (*Solanum lycopersicum*) plants infested with the greenhouse whitefly *Trialeurodes vaporariorum* was determined within a 14 days period. The VOC emission profiles varied concomitantly with the duration of whitefly infestation. A total of 36 different VOCs were detected during the experiment, 26 of which could be identified: 23 terpenoids, plus decanal, decane, and methyl salicylate (MeSA). Many VOCs were emitted exclusively by infested plants, including MeSA and 10 terpenoids. In general, individual VOC emissions increased as the infestation progressed, particularly at 7 days post-infestation (dpi). Additional tunnel experiments showed that a 3 days exposure to VOC emissions from whitefly-infested plants significantly reduced infection by a biotrophic bacterial pathogen. Infection of VOC-exposed plants induced the expression of a likely tomato homolog of a methyl salicylate esterase gene, which preceded the expression of pathogenesis-related protein genes. This expression pattern correlated with reduced susceptibility in VOC-exposed plants. The observed cross-kingdom effect of plant-plant signaling via VOCs probably represents a generalized defensive response that contributes to increased plant fitness, considering that resistance responses to whiteflies and biotrophic bacterial pathogens in tomato share many common elements.

Keywords Greenhouse whitefly · Methyl salicylate esterase · Plant-plant communication · Priming · *Trialeurodes vaporariorum* · Volatile organic compounds

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Introduction

Trialeurodes vaporariorum (Westwood) (Hemiptera: Aleyrodidae), also known as the greenhouse whitefly, is an economically important field and greenhouse pest of horticultural and ornamental crops (Inbar and Gerling, 2008). Whiteflies are generalist and highly polyphagous insect pests that cause considerable damage to crops by direct phloem feeding that leads to reduced plant vigor, plant stunting, foliage deformation, and/or discoloration or defoliation (Berlinger, 1986). Damage also can be caused indirectly by the transmission of plant viruses (Jones, 2003). Reduced photosynthesis also can be caused by light blockage resulting from fungal growth promoted by honeydew excreted by the whiteflies (Byrne and Miller, 1990). Several physiological disorders are known to occur in silverleaf whitefly-infested plants (McCollum et al., 2004 and

references therein). Compared with other feeding guilds, phloem-feeders cause minimal mechanical damage to plants, although saliva components, mechanical damage, and endosymbiotic-borne cues are believed to contribute to the induction of plant defense responses against whiteflies (Inbar and Gerling, 2008). These resemble plant responses to biotrophic pathogen infection, activated via salicylic acid (SA), and are characterized further by the repressed expression of several jasmonic (JA)-, ethylene-, and photosynthesis-related genes (Walling, 2008 and references therein; Zhang et al., 2009). Additional findings have shown that whitefly infestation in tomato produces a distinctive phase-specific expression pattern of several genes associated predominantly with photosynthesis, senescence, secondary metabolism, and biotic stress that is dependent on the degree of larval feeding (Estrada-Hernández et al., 2009). A recent report also showed that the temporal and spatial expression of wound- and defense-related genes in tomato is similar in *Bemisia tabaci* biotype B- and *T. vaporariorum*-infested plants (Puthoff et al., 2010).

Recent studies have indicated that *B. tabaci* apparently uses specific plant volatiles, in addition to visual cues, for the initial selection of a host (Isaacs et al., 1999 and references therein; Bleeker et al., 2009). This is in accordance with data demonstrating that plant volatile organic compounds (VOCs) play a role in enabling insects to recognize host plants from a distance. Plant VOCs that are released in response to herbivory also can be a determining factor in the attraction of predators and parasitoids (recently reviewed by Arimura et al., 2010). Additionally, they can play a role in the direct defense against herbivores and pathogens, to the extent that the use of VOCs as insect repellents of natural origin has been proposed as a potential alternative to chemical pesticides (Sánchez-Hernández et al., 2006; Bleeker et al., 2009 and references therein). However, VOCs might also have a negative impact on emitting plants, as they may be used as signals by parasitic plants and herbivorous insects to locate hosts (reviewed in Heil and Karban, 2010) or as allelopathic agents causing plant growth inhibition (reviewed by Arimura et al., 2010). Furthermore, a plant VOC-related repelling effect on beneficial predatory insects has been reported recently in *Arabidopsis* (Snoeren et al., 2010).

The majority of plant volatiles are derived from the isoprenoid pathway, which appears to be regulated by the octadecanoid pathway that controls the biosynthesis of JA (Ament et al., 2004; Sánchez-Hernández et al., 2006). In addition to JA, further experimental data also suggest that the regulated synthesis of herbivore-induced plant VOCs is dependent on signaling pathways controlled by ethylene and Ca^{2+} (reviewed by Arimura et al., 2011). The participation of systemin in the induction and regulation of indirect plant defense responses involving VOCs also has been proposed (Corrado et al., 2007).

Another important aspect of VOCs is that their emission from several species of plants subjected to natural or imposed herbivory, viral infection, or mechanical damage triggers defensive responses in neighboring undamaged plants. These may be taxonomically related or not (reviewed by Heil and Karban, 2010; Arimura et al., 2010). The phenomenon has been reported predominantly in plant–herbivore interactions, although a few reports indicate that VOCs may also induce pathogen resistance (Shulaev et al., 1997; Kishimoto et al., 2005). In some cases, plants exposed to VOCs show no noticeable changes in their defense levels, but are nevertheless able to mount a stronger and faster response when challenged by attacking herbivores (Frost et al., 2008 and references therein; Ramadan et al., 2011), or pathogens (Yi et al., 2009 and references therein). This mechanism, termed "priming", allows plants to respond more rapidly and effectively to subsequent attack without a costly investment in direct resistance induction (Frost et al., 2008; Goellner and Conrath, 2008).

In the present study, we chemically analyzed VOCs emitted by tomato plants infested with *T. vaporariorum* during a 14 days period, and studied the effect of these VOCs on pathogen resistance of a VOC-exposed undamaged tomato plant. The VOCs detected were mostly terpenoids, and gradually increased in abundance until reaching a peak at 7 days post-infestation (dpi). The emission rates of VOCs were relatively stable, as most compounds were still abundant at 14 dpi. Biologically active signals like ocimene, MeSA, and decanal were detected in the VOC emissions from whitefly-infested plants. Tomato plants exposed to VOCs from whitefly-infested plants showed increased resistance to infection by a virulent bacterial pathogen and also presented augmented expression of a tomato homolog of a methyl salicylate esterase gene and two other typical defense marker genes. The role played by VOCs in plant-to-plant communication and the way they appear to influence the plant's response to enemies of two different kingdoms (herbivorous insects, phytopathogens) will be discussed.

Methods and Materials

Plants and Insects Tomato plants (*Solanum lycopersicum* cv. Rio Fuego; Cal-Oro Vegetable Seeds, United Genetics, Inc., Gilroy, CA,USA) were grown in 750 ml pots using a rich soil mixture. The plants were watered daily and fertilized weekly with a 20–10–20 (N–P–K) soil drench solution (Peters Professional; Scotts-Sierra Horticultural Products, Marysville, OH, USA). They were maintained in a conditioned room under controlled conditions of light (≈ 300 $\mu mol\,m^{-2}sec^{-1}$), temperature and photoperiod (16:8, L:D, at a constant temperature of 27 °C). Four-to-5-week-old plants having 5–6 expanded leaves were used in all

experiments. Virus-free whiteflies (*Trialeurodes vaporariorum*) were obtained from a laboratory colony reared on tomato in another conditioned room operating under the same conditions as described above. Positive identification of the whitefly colony as *T. vaporariorum* was performed by using samples of 1st, 2nd, and 3rd instar larvae, puparia, and adults, following the procedures described by Martin (1987). All infestation assays were performed in the same conditioned room and were conducted using 1- to-5-day-old adult whiteflies.

Volatile Analyses For *T. vaporariorum* infestations, five plants were confined inside the colony-harboring cages for 2, 5, 7, 9, 11, or 14 days. In all cases, approximately 500 whiteflies were observed in each infested plant during volatile collection, which showed no evident signs of damage, such as chlorotic or necrotic lesions, even after prolonged infestation. At the end of each whitefly infestation time-point, plants were enclosed in volatile-emitting-inert plastic bags (Toppits®, Minden, Germany) followed by the insertion of a Stable Flex Solid Phase Micro-Extraction (SPME) fiber (2 cm, Carboxen/Polydimethylsiloxane/Carbowax; Supelco, Bellefonte, PA, USA) for volatile collection. Fibers were exposed for a period of 18 hr and then desorbed for 30 sec directly into the gas chromatograph (GC) injector (180 ° C). The VOCs were analyzed by GC-mass spectrometry (MS) employing a Hewlett Packard (HP) GC 5890-MS 5972 system (Agilent Technology, Palo Alto, CA, USA). Analytes were separated by using an HP-free fatty acid phase (FFAP) capillary column (30 m long, 0.32 mm diam., and 0.5 μm phase thickness). The temperature program used for analysis was as follows: initial temperature at 60 °C, which was increased to 80 °C at 5 °C/min; having reached 80 °C and after a 1 min hold, the temperature was increased to 210 °C at 8 °C/min and maintained for 5 min. Compounds were identified using the National Institute of Standards and Technology (NIST) mass spectral library and, when available, verified by authentic standards (Fluka Chemie, Steinheim, Germany; Sigma-Aldrich, St Louis, MO, USA; see Table 1). The Kovats retention index (KI) for each compound also was calculated using an alkane mixture (Sigma-Aldrich). Special care was taken to ensure equal conditions during sampling. Blank analyses of the plastic bags were run before starting. The volatiles from 5 replicates of each treatment were collected at each of the time points examined. Results were expressed as mean percentages obtained by peak area normalization.

Plant-to-Plant Communication Experiments Experiments were conducted in an acrylic tunnel placed inside a greenhouse kept under natural conditions of light and temperature. The tunnel (2 m×65 cm×65 cm), was divided into six individual equal compartments (67×32.5×65 cm) by a longitudinal 2 m acrylic division placed exactly at the middle of the tunnel. Further divisions on each side were made by placing two equidistant transversal panels covered with antivirus/no-thrips screens (mesh size: 50×24; BioQuip Products, Inc., Gardena, CA, USA). Both ends of the tunnel were similarly covered with antivirus/no-thrips screens. This design enabled the free flow of air between the compartments and kept the plants otherwise isolated from the exterior. In the first phase of the experiment, two groups of 6 plants were infested by placing them inside the cages housing the whitefly colony for 24 h. The plants then were confined in two cages covered with antivirus/no-thrips screens, dimensioned to precisely fit in the tunnel compartments. Infested plants were placed in the central compartments and remained there for 5 days. At the 6th day, all four lateral compartments were filled with 6 undamaged plants each and were exposed to the VOCs from the whitefly-infested plants for an additional 3 days (Fig. 1). Control experiments were performed identically, except that uninfested plants were placed at the central compartments. After this period, infested/control plants were removed, and neighboring plants exposed to VOCs were spray-inoculated directly in the tunnel with 1 ml each of bacterial suspensions of *Pseudomonas syringae* pv tomato DC3000 grown on MG medium and subsequently adjusted to an OD of 0.25 in a 50 mM phosphate buffer, pH 7.0. The plants were covered with plastic bags immediately after inoculation and remained covered for 3 days to facilitate the infection process. At this point (3 days after inoculation, dai), the bags were removed, and leaf sampling was initiated to assess bacterial populations. Leaf samples (1 leaf per plant) from 4 plants were taken at 3, 5, 6, 7, 8, and 9 dai. Details regarding routine culture conditions and *in planta* bacterial growth assays are described elsewhere (Valenzuela-Soto et al., 2011). Plant-to-plant communication experiments were performed three times with consistent results. Additional experiments were performed to obtain samples for the gene expression assays. In these experiments, the sampling procedure differed slightly from the above; leaf samples for qPCR (see below) were sampled at 1, 3, and 5 dai.

Gene Expression Analysis by qPCR Leaf RNA was isolated using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) treated with DNAase and re-purified with the RNeasy kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocols. After RNA extraction, 2 μg were reverse transcribed with SuperScript II (Invitrogen) by using oligo-$dT_{(12–18)}$ primers. Real-time PCR amplifications were performed in 48-well-plates using SYBR Green detection chemistry in a StepOne™ Real-Time PCR System (Applied Biosystems/Ambion, Austin, TX, USA). Reactions were prepared in a total volume of 8 μl containing: 1 μl of first strand cDNA template, 1.6 μl of forward and reverse primers (2 μM), 4 μl of Fast SYBR® Green Master Mix

Table 1 Time course of VOCS emitted by *Trialeurodes vaporariorum*-infested tomato plants (as % of total peak area)

Compounds	KI	Control	Infestation time [d]					
			2	5	7	9	11	14
(+)-α-pinene*	1034	1.83±0.55^{a}	0.53±0.15^{abc}	0.98±0.16^{ac}	0.24±0.04^{abc}	1.25±0.26^{a}	traces^{bc}	traces^{bc}
(+)-2-carene*	1133	3.49±0.56^{a}	2.88±1.01^{a}	0.68±0.11^{b}	1.75±0.46^{ab}	2.23±0.61^{ab}	2.98±0.32^{a}	0.78±0.17^{b}
(*R*)-α-phellandrene*	1178	2.37±0.37	1.39±0.32	3.23±1.11	3.25±0.59	2.23±0.41	2.09±0.25	2.48±0.17
(*R*)-(+)-limonene*	1203	29.30±2.25^{a}	10.91±1.80^{a}	7.51±0.96^{b}	7.18±0.67^{b}	8.89±1.07^{b}	6.68±0.83^{b}	9.21±3.24^{b}
β-phellandrene	1213	33.05±8.30^{a}	16.88±2.25^{ab}	12.18±4.35^{b}	16.58±1.19^{ab}	22.09±3.95^{ab}	24.29±3.05^{ab}	26.55±3.93^{ab}
(*E*/*Z*)-β-ocimene*	1263	0.00^{c}	0.49±0.08^{b}	0.49±0.16^{b}	2.12±0.48^{a}	0.78±0.27^{b}	1.08±0.22^{ab}	0.95±0.19^{ab}
terpinolene*	1297	0.00	1.34±0.65	0.00	0.00	0.00	0.00	0.00
(*E*/*Z*)-(+)-limonene oxide*	1468	1.13±0.47	0.00	0.00	0.00	0.00	0.00	0.00
δ-elemene	1474	4.01±0.98	3.80±1.09	4.98±0.95	5.01±1.37	2.28±0.70	4.28±1.00	5.33±1.33
α-copaene	1490	1.48±0.46	1.54±0.28	0.82±0.20	1.78±0.56	2.06±0.73	2.49±0.24	2.11±0.35
decanal*	1510	1.61±0.70	1.26±0.31	1.41±0.33	2.66±0.39	1.53±0.34	2.92±0.50	3.13±0.38
aristolene	1579	1.37±0.63^{ab}	1.98±0.36^{ab}	0.82±0.13^{b}	2.17±0.64^{ab}	1.29±0.31^{ab}	2.78±0.58^{a}	2.02±0.39^{ab}
β-caryophyllene*	1599	7.32±1.59^{b}	19.36±4.19^{ab}	26.68±3.01^{a}	18.46±2.64^{ab}	15.43±2.99^{ab}	15.47±2.16^{ab}	15.93±2.55^{ab}
α-gurjunene	1611	0.00^{c}	2.29±0.40^{ab}	0.81±0.16^{bc}	2.16±0.25^{ab}	1.80±0.36^{ab}	1.74±0.15^{ab}	2.55±0.69^{a}
NI-1	1619	0.00^{a}	2.81±0.93^{ab}	0.00^{a}	3.03±0.32^{b}	2.15±0.44^{ab}	2.43±0.33^{ab}	2.16±0.21^{ab}
NI-2	1647	0.00	0.89±0.19	0.63±0.07	0.00	0.00	0.00	0.00
α-muurolene	1673	0.00c	2.69±0.31^{ab}	2.07±0.38^{bc}	1.78±0.51^{bc}	1.91±0.72^{bc}	4.39±0.71^{a}	3.24±0.50^{ab}
α-caryophyllene*	1678	2.67±0.77^{b}	4.08±0.84^{ab}	8.95±1.68^{a}	5.26±0.64^{ab}	3.95±0.81^{ab}	5.48±1.07^{ab}	6.12±1.34^{ab}
isoledene	1684	0.00^{a}	1.49±0.19^{ab}	1.05±0.26^{ab}	2.79±0.21^{b}	1.57±0.58^{ab}	traces^{a}	traces^{a}
NI-3	1690	2.00±0.70	1.68±0.24	1.72±0.24	1.93±0.51	2.33±0.52	1.96±0.28	1.65±0.19
δ-selinene	1704	0.00^{a}	1.83±0.20^{b}	2.00±0.39^{b}	2.64±0.77^{b}	1.85±0.75^{b}	traces^{a}	traces^{a}
germacrene D*	1722	2.90±1.24^{a}	2.42±0.44^{ab}	0.00^{b}	4.28±0.86^{a}	3.53±0.64^{a}	3.09±0.36^{a}	3.35±0.32^{a}
NI-4	1732	2.15±0.59^{ab}	1.75±0.23^{ab}	1.05±0.25^{b}	3.18±0.28^{a}	2.31±0.57^{ab}	1.99±0.21^{ab}	1.72±0.17^{ab}
(+)-aromadendrene*	1743	0.00^{c}	2.15±0.34^{ab}	2.50±0.49^{ab}	0.84±0.38^{bc}	2.61±0.31^{ab}	3.45±0.50^{a}	3.61±0.66^{a}
δ-cadinene	1781	0.00^{c}	1.98±0.26^{b}	1.43±0.20^{bc}	2.19±0.77^{b}	5.13±0.78^{a}	traces^{c}	traces^{c}
β-elemene	1798	2.70±0.82	3.86±0.73	2.04±0.50	4.32±0.95	3.75±0.99	3.34±0.70	2.88±0.42
decane*	1000	0.00^{b}	1.77±0.16^{a}	2.69±0.79^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}
methyl salicylate*	1814	0.00^{a}	0.00^{a}	3.98±0.53^{b}	3.18±0.83^{ab}	3.93±1.05^{ab}	4.18±1.56^{b}	4.97±0.84^{b}
NI-5	1817	0.00^{b}	1.34±0.19^{a}	0.00^{b}	1.91±0.46^{a}	1.93±0.36^{a}	0.00^{b}	0.00^{b}
NI-6	1831	0.00^{a}	1.45±0.15^{ab}	5.40±2.05^{b}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
γ-elemene	1852	2.54±0.90^{a}	1.67±0.21^{a}	1.80±0.40^{a}	1.93±0.48^{a}	1.57±0.45^{ab}	traces^{ab}	0.00^{b}
NI-7	1861	0.00^{c}	1.54±0.09^{a}	0.85±0.13^{b}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}
(−)-caryophyllene oxide*	2007	0.00^{b}	2.05±0.33^{a}	1.76±0.28^{a}	0.85±0.48^{ab}	1.67±0.76^{ab}	2.86±0.89^{a}	1.92±0.50^{a}
(*E*/*Z*)-nerolidol*	2047	0.00	1.02±0.21	0.75±0.17	0.00	0.00	0.00	0.00

Compounds in the table are ordered according to their calculated Kovats index (IK). Means in the same row that are followed by different letters represent statistically significant differences among infestation times [d](ANOVA-Tukey-Kramer or nonparametric analyses, $P \leq 0.0$, $\alpha = 0.05$). Each mean represents data from 5 replicates. Traces = detected content is ≤0.005 %.

* Identified by comparing to authentic compounds

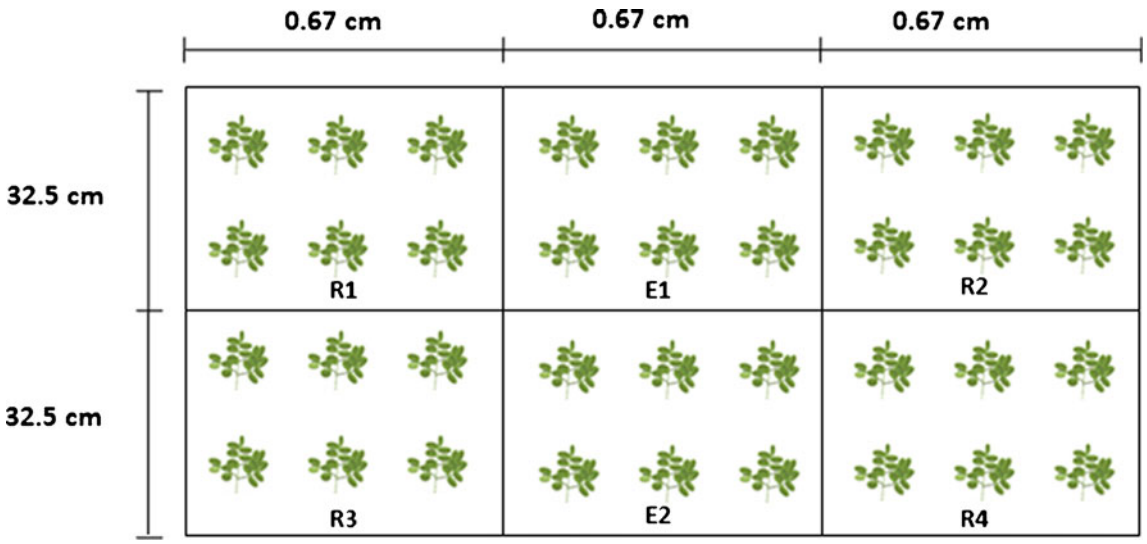

Fig. 1 Experimental set-up of the tunnel experiments performed to test plant-plant communication via volatiles between tomato plants infested with *Trialeurodes vaporariorum* (chambers E1 and E2) and neighboring non-infested plants (chambers R1 to R4). Two groups of 6 infested plants were placed in the central E and E2 compartments of the tunnel, respectively, whereas four groups of 6 intact plants were placed in the remaining compartments (R1 to R4). The compartments were isolated longitudinally; the transversal divisions that were covered with anti-virus/no-thrips screens and placed between them permitted the free flow of air from the exterior. No means to accelerate the air flow through the tunnel were employed. Sampling of leaf material at each time point involved at least one plant from each individual compartment. In control experiments, uninfested plants were placed in chambers E1 and E2

(Applied Biosystems), and 1.4 μl of sterile de-ionized-distilled water. Gene-specific primers were used for the amplification of the defense-related *PR-1* and *GLU* genes, encoding the PR1 protein and an acidic β-1, 3-glucanase isoform as described previously (Song et al., 2011). A likely tomato methyl esterase (*SlMES*) was amplified using forward, 5′CATGGAGGTTGGTGTTTGGTA3′, and reverse, 5′GCCAAATCAAGAGTTGTGACC3′, specific primers. These primers were designed based on sequence similarity with a cDNA sequence of tomato (GeneBank No. AK322288.1; Aoki et al., 2010) and potato methyl esterase1 (GeneBank no. CK270870.1, Manosalva et al., 2010). Elongation factor 1-α (*EFα1*) and a *TIP41*-like family gene (*TIP41*) (Expósito-Rodríguez et al., 2008) were examined as endogenous control genes. The cycling conditions were set as follows: initial denaturation step at 95 °C for 10 min to activate the AmpliTaq Gold DNA Polymerase, followed by 40 cycles of denaturation at 95 °C for 15 sec and annealing at 58 °C for 1 min. At the end of PCR amplification, a melting curve analysis was performed immediately to confirm the specificity of the reactions. Baseline and threshold cycles (Ct) were automatically determined using Real-Time PCR System software. PCR reactions were run in duplicate for each sample (N=4). PCR efficiencies for all genes tested were greater than 95 %. Relative expression was calculated using the comparative cycle threshold method (Livak and Schmittgen, 2001), where delta (Δ) cycle threshold of cDNA from controls was defined as 100 % transcript presence. Transcript abundance data were normalized against the average transcript abundance of two endogenous control genes, elongation factor 1-α (*EFα1*) and a *TIP41-like* gene (*TIP41*) as determined during the assays. The fold change in expression of the target genes in each treatment was calculated using the following equation: $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct = (Ct\ target\ gene - average\ Ct\ reference\ genes)_{treatment} - (Ct\ target\ gene - average\ Ct\ reference\ genes)_{control}$.

Statistical Analysis Differences in individual volatile emissions among infestation times were statistically analyzed using analysis of variance (ANOVA) followed by a Tukey–Kramer *post-hoc* test by using a JMP® version 3.2 software package (SAS Institute, Cary, NC, USA). When necessary, data followed a Johnson transformation (Chou et al., 1998) prior to analyses, or if transformation failed, data were analyzed via a Kruskal-Wallis test. Differences among infestation time points were assessed with a principal component analysis (PCA) using IBM® SPSS® Statistics 19. In the plant-to-plant communication assay bacterial populations were compared between plants exposed to VOCs using *t-tests*. *Before t*-tests were performed, data followed a Box-cox transformation (Sakia, 1992) to meet the assumptions of normality. Data of the gene expression analyses were compared using a one-way ANOVA followed by a Tukey–Kramer *post-hoc* test. Data followed a Johnson transformation to meet the assumptions of normality. Statistical evaluations of the plant-to-plant communication experiments and the gene expression analyses were performed using Mini-Tab® 15 and Statistica® 8 software.

Results

VOC Emissions in T. vaporariorum-Infested Plants The emission of VOCs from *T. vaporariorum*-infested plants varied among different time intervals of the 14 days period. The emission rates of the majority of the constitutive tomato VOCs, excluding β- and δ-elemene, α-phellandrene, α-copaene, and decanal, significantly changed in response to *T. vaporariorum* infestation. Overall, the VOCs detected followed three emission patterns: 1) 26.47 % of VOCs showed no significant variation; 2) 44.11 % were not detected in controls but were detected after a minimum time of infestation, with levels increasing thereafter; 3) 29.42 % showed variation (increase or decrease) at some time during infestation. These results are summarized in Table 1. The presence of β-phellandrene, (*R*)-(+)-limonene, β-caryophyllene, δ-elemene, and (+)-2-carene, is in accordance with reported data (Buttery et al., 1997).

Some inducible VOCs were detected only at 2 and 5 dpi, suggesting a fast and short-lived early response (e.g., nerolidol, decane, terpinolene, NI-2, NI-6, and NI-7; all disappearing between 2 and 5 dpi). Several VOCs showed a significant and relatively late increase above control levels that occurred at ≥7 dpi (e.g., α-muurolene, isoledene, (+)-aromadendrene, and δ-cadinene). The emission of some VOCs such as α- and β-caryophyllene, reached ≥ three-fold higher levels than control plants, in at least one time point during *T. vaporariorum*-infestation. In contrast, (*E*/*Z*)-(+)-limonene oxide and γ-elemene ceased to be emitted at detectable levels in infested plants. Others, like germacrene D and NI-5 showed an erratic pattern of emission: emission of germacrene D decreased to undetectable levels at 5 dpi only to resurge at 7 dpi, and NI-5 sporadically appeared at 2, 7, and 9 dpi. In contrast, the presence of aristolene, a volatile component produced by rhizomes of medicinal Chinese plants (Tanaka and Komatsu, 2008) has not been previously reported, to our knowledge, as a component of the tomato plant aroma.

VOCs known to have biological activity in terms of indirect defense responses leading to pathogen or herbivore resistance or to the recruitment of pest's parasites and predators (i.e., ocimene and MeSA) were detected after *T. vaporariorum*-infestation. The induction of (*E*/*Z*)-β-ocimene was detectable at 2 dpi, reaching maximum emission levels at 7 dpi, while MeSA was not detected until 5 dpi, increasing steadily thereafter until 14 dpi.

The analysis of 34 VOCs varied significantly among infestation periods. Principal component 1 explained 29.26 % of total variation, while 20.98 % of variation was explained by principal component 2. Component 1 was associated mainly with α-gorjunene, aristolene, germacrene D, (*E*/*Z*)-β-ocimene, decanal, NI-7, and NI-4. Component 2 was related mainly to NI-6, nerolidol, decane, NI-7, NI-2, δ-elemene, and δ-selinene. A plot based on the first and second PCA axes revealed a clear separation of the emission patterns from control plants and plants in early infestation periods (i.e., 2 and 5 dpi) and late infestation periods (i.e., 7, 9, 11, and 14 dpi), suggesting early and late VOC emission profiles in response to *T. vaporariorum* infestation (Fig. 2).

Increased Disease Resistance in Plants Exposed to VOCs Neighboring intact plants exposed for 3 days to VOCs emitted from plants that had been infested for 6 days with *T. vaporariorum* showed a significantly increased resistance to bacterial infection by virulent *P. syringae* pv. *tomato* DC3000, as shown by the results presented in Figure 3. The protective effect was noticeable from the early stages of the infective process, since bacterial numbers were already significantly lower in plants exposed to *T. vaporariorum*-infested plants as compared to those exposed to control plants at 3 dai (t=3.466; 20 *df*; P=0.002), and remained so until 9 dai (t=5.651; 28 *df*; P<0.001). The decrease observed in control bacterial populations that was observed consistently at this last time point remains unexplained. The exposure to infested plants was timed to coincide with the beginning of active MeSA emissions in infested plants, as determined previously (see Table 1) and was long enough to ensure a functional and durable priming response in the receiving plants, as determined from several other priming experiments reported before (Frost et al., 2008; Yi et al., 2009; Heil and Adame-Álvarez, 2010; Ramadan et al., 2011).

Gene Expression Assays Gene expression analysis of *PR1* and *GLU* pathogen defense marker genes was performed in plants exposed to VOCs emitted by *T. vaporariorum*-infested and control plants exposed to intact plants. The expression of *SlMES*, a likely homolog of *AtMES1*, a methyl salicylate esterase gene identified in *Arabidopsis* (Vlot et al., 2008), which shares similarities with other methyl esterase genes in potato and tobacco (Manosalva et al., 2010), also was included in the analysis. This was done in order to explore the possibility that the conversion of MeSA to SA, a central SAR-eliciting hormone, might have contributed to the resistance observed. MeSA was detected in the blend of VOCs released by infested plants. Significant differences were found when relative fold differences were compared among days after infection for the *PR-1* (F=25.22; 2 *df*; P<0.001), *GLU* (F=29.60; 2 *df*; P<0.001) and *SlMES* (F=7.59; 2 *df*; P=0.011) genes (Fig. 4). Neighboring plants exposed to VOC emissions from whitefly-infested plants responded to bacterial infection with a rapid induction of *SlMES* and *GLU*, at 1 dpi, with a slower induction of *PR1* being observed until 5 dpi. At this point, the expression of *GLU* was particularly strong. A time lapse in the expression of these genes was observed at 3 dpi. Experiments performed with neighboring plants exposed to VOCs from both infested and uninfested plants but not challenged with the bacterial pathogen showed no changes in the expression levels of these genes (results not shown).

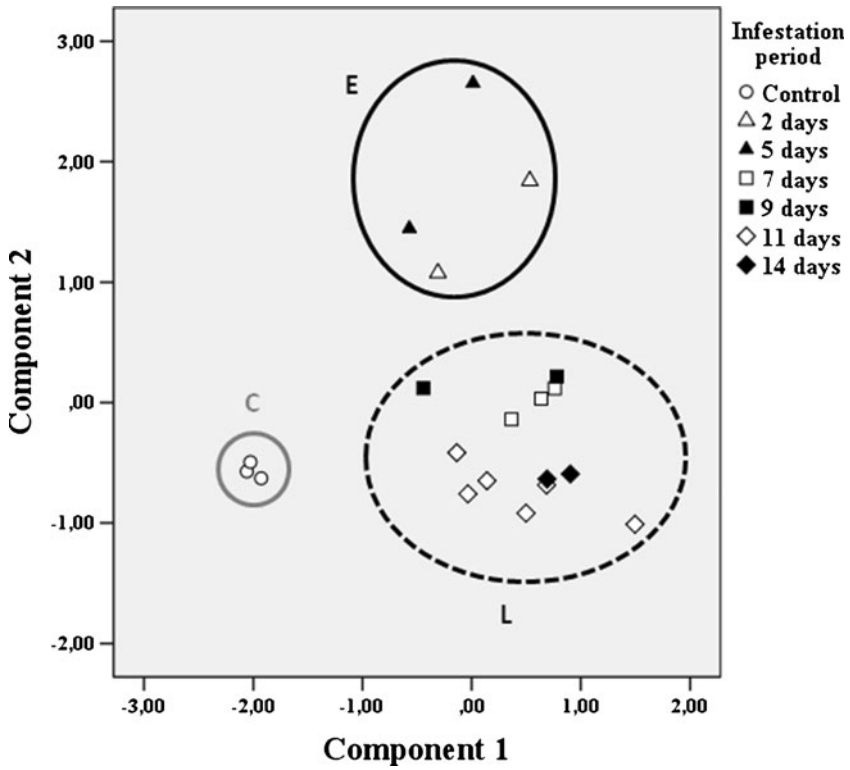

Fig. 2 PCA sample plot of the emission pattern of 34 VOCs generated by *Trialeurodes vaporariorum*-infested plants at several time points. C = control (uninfested) plants; E = early infestation time points (2 and 5 dpi) and L = late infestation time points (7, 9, 11, and 14 dpi)

Discussion

The work here described presents the analysis of VOCs emission from *T. vaporariorum*-infested plants and presents evidence that plant-plant signaling affects resistance across kingdoms as plants exposed to VOCs emitted by herbivore-infested conspecifics showed increased resistance to a bacterial pathogen. The data presented resemble those of a previous study showing that lima bean plants exposed to volatiles emitted from BTH-treated conspecifics were more resistant to bacterial infection (Yi et al., 2009).

Tomato plants significantly increased their volatile emission during *T. vaporariorum* infestation, similarly to previously reported data for plants infested by the whiteflies *B.*

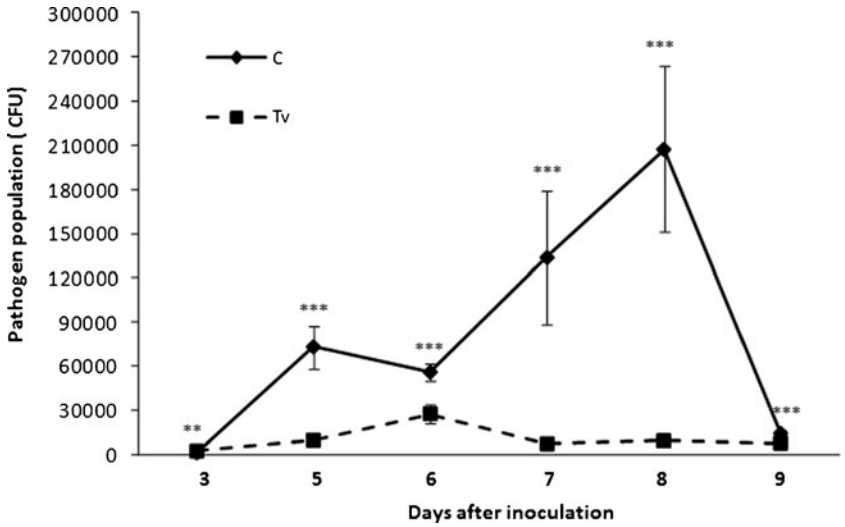

Fig. 3 Airborne resistance of tomato plants exposed to volatiles emitted by conspecifics infested with the greenhouse whitefly and subsequently challenged with a bacterial pathogen. After a 3 d exposure to volatile emission from plants infested with *Trialeurodes vaporariorum* (Tv) or uninfested control plants (C), plants were spray-inoculated with *Pseudomonas syringae* pv. *tomato* DC3000. Bacterial populations (registered as CFUs) were assayed in leaves (N=4) sampled at 3, 5, 6, 7, 8, and 9 d after infection (dai). Each point indicates the mean ± SE. Asterisks indicate significant differences between *Trialeurodes vaporariorum* exposed and C plants at * $P \leq 0.05$ or ** $P \leq 0.01$). The experiment was repeated 3 times with similar results

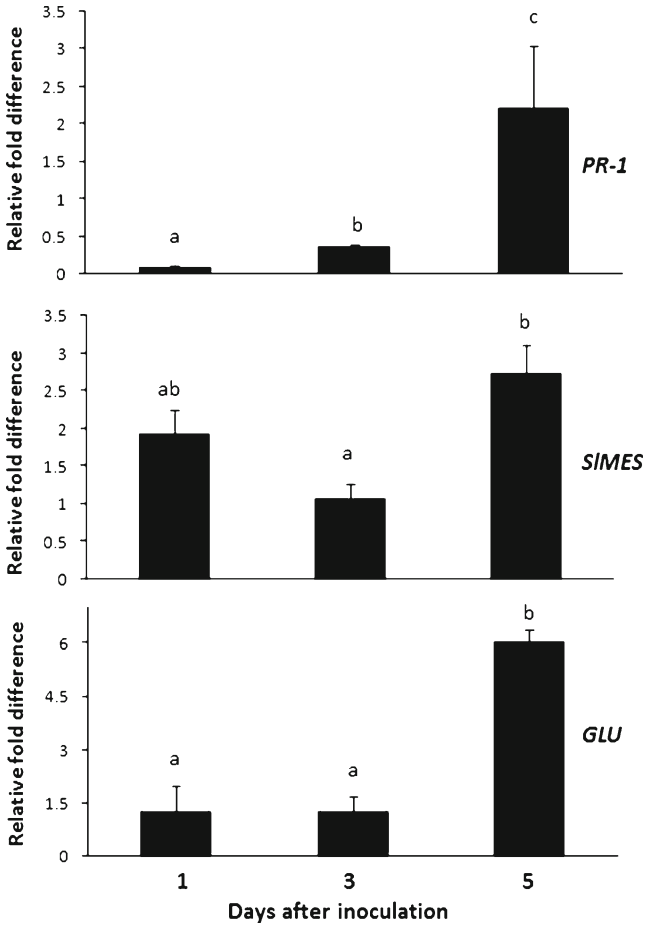

Fig. 4 Time course expression of the Pathogenesis Related Protein-1 (*PR1*), tomato methyl esterase (*SlMES*) and acidic β-1,3-glucanase (*GLU*) genes as determined by qRT-PCR. Expression was measured in tomato neighboring plants exposed to volatiles emitted by conspecifics infested with the greenhouse whitefly *Trialeurodes vaporariorum* and subsequently challenged with the bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000. Leaf samples were taken at different d after pathogen inoculation as described. All values are normalized to the uninfected control. Bars indicate means ± SE (N=4) and different letters represent statistically significant differences in the expression of a given gene within the time frame of the experiment ($P \leq 0.05$)

tabaci and *B. argentifolii* (Rodríguez-Saona et al., 2003; Stansly and McKenzie, 2007; Bleeker et al., 2009). The plant's response to whiteflies has been interpreted as being part of a plant defense mechanism designed to positively influence the foraging behavior of the whitefly parasitoid *Encarsia formosa* (Birkett et al., 2003; Stansly and McKenzie, 2007). No green leaf volatiles (GLVs) were detected in the VOCs emission from *T. vaporariorum*-infested plants (Table 1), probably as a consequence of the time frame of VOCs collection and/or lack of perceptible mechanical damage usually associated with phloem feeding whiteflies (Walling, 2008). Conversely, the VOC composition in the emissions released by *T. vaporariorum*-infested tomato plants (Table 1) was similar to the one detected in spider mite-infested tomato plants (Kant et al., 2004), even though these phytophagous arthropods belong to different feeding guilds and different experimental conditions were employed.

Eight monoterpenes were identified in the tomato VOC mixture, including β-phellandrene and (*R*)-(+)-limonene (Table 1). They constituted more than 70 % of the volatile mixture detected in intact tomato plants. In contrast, sesquiterpenes constituted 50 % of total VOCs in infested plants, where α- and β-caryophyllene reached significantly higher emission levels than controls in infested plants at 5dpi. The latter was once considered as the basis of a non-destructive method for the detection of *Botrytis cinerea* in infected tomato plants (Jansen et al., 2009). The above results were in agreement with those in several other studies that have shown increased volatile terpene emission from damaged tomato plants (Sánchez-Hernández et al., 2006 and references therein; Bleeker et al., 2009). Also important were seven unidentified compounds, three of which (NI-1 to −3) were tentatively considered as sesquiterpenes. The finding that some of the unidentified VOCs were detected only in VOC emissions from infested plants (i.e., NI-1and −2 and NI-5 to −7) suggests their potential role in indirect plant defense and/or plant-plant communication. Taking into consideration the feeding habit of *T. vaporariorum*, which causes minor disruption of plant tissues, it is also conceivable that the significant accumulation of volatile terpenes detected in response to whitefly infestation was the result of *de novo* biosynthesis rather than of a passive release from severed glandular trichomes, which are rich reserve tissues for these compounds in tomato plants (Colby et al., 1998).

The presence of decanal in the VOC blend of intact tomato was similar to findings in poplar (Hu et al., 2009 and references therein), although the biogenic biosynthetic process in tomato is unknown. On the other hand, the induced emission of decane in infested plants was unexpected. This compound has been reported predominantly only as a component of bacterial odor leading to induced systemic resistance and growth promotion in *Arabidopsis* (Ryu et al., 2004) and as a component of volatile blends of hemiparasite plants (Troncoso et al., 2010). The possible role of both decanal and decane in direct and/or indirect defense responses in tomato plants remains to be determined.

Interestingly, MeSA was detected only in the VOC mixtures emitted by *T. vaporariorum*-infested tomato plants. MeSA is a ubiquitous VOC, consistently reported in volatile emissions induced by herbivore damage in several plant species by a diverse array of pests (James, 2003 and references therein; Birkett et al., 2003; Kant et al., 2004). The increase in MeSA levels, which occurred concomitantly with increasing duration of *T. vaporariorum* infestation in tomato, was consistent with recent data showing that the intensity of aphid-induced MeSA emissions in boreal forest

trees depended on the duration of aphid infestation (Blande et al., 2010 and references therein). According to these studies, MeSA emissions occur only after aphid feeding exceeds a still undetermined threshold level. This also could explain the relatively tardy emission of MeSA observed in *T. vaporariorum*-infested tomato.

Increased MeSA emissions in whitefly-infested plants could play a defensive role by its ability to attract foraging predators and parasitoids. This has been demonstrated in many field and laboratory experiments that show the attractive effect of MeSA on beneficial insects of tomato, strawberry, and other crops (Stansly and McKenzie, 2007; Lee, 2010 and references therein). Another possibility is that MeSA could have contributed to the induced resistance to bacterial infection observed in tomato plants exposed to VOCs emitted by whitefly-infested plants (Fig. 3). Studies performed in tobacco and *Arabidopsis* have provided evidence suggesting that MeSA could be one of the mobile signals needed to trigger systemic acquired resistance (SAR) to pathogen infection (Liu et al., 2011 and references therein). However, contradictory data question the role of MeSA in SAR and suggest that its production might be a mechanism to attenuate resistance by volatilizing SA (Attaran et al., 2009). The induction of defense-related genes in neighboring plants also has been attributed to MeSA (Shulaev et al., 1997). Moreover, MeSA is one of the few VOCs for which an enhancing effect on plant disease resistance has been demonstrated in addition to a few GLVs (reviewed by Yi et al., 2010). More experimental work obviously is needed to determine the role of MeSA as an active airborne signal that leads to increased resistance in tomato. However, the rapid induction of a likely tomato MeSA esterase gene homolog in infected plants previously exposed to volatiles emitted from *T. vaporariorum*-infested plants could be an indirect indication that an esterase activity might be needed to convert the MeSA volatile signal to SA *in planta* in order to trigger SAR, similarly as in studies performed in tobacco, *Arabidospis*, and potato (Vlot et al., 2008; Park et al., 2009; Manosalva et al., 2010). However, such a scenario requires proof that: i) neighboring intact tomato plants are capable of adsorbing and accumulating MeSA from emitter VOCs, similarly to what has been observed in lima bean and other plants (Choh et al., 2004); and ii) SA is directly inducing defensive responses in receiving plants after its release from MeSA by an active MeSA esterase enzyme. Irrespective of the possible role of SA derived from the enzymatic hydrolysis of MeSA, it is clear that the increased expression levels of the two PR genes examined, in particular *GLU*, correlated with the resistance to virulent *P. syringae* infection observed in volatile-exposed intact plants (Fig. 4). A similar behavior has been observed in lima bean plants exposed to volatile blends enriched in nonanal and MeSA emitted by BTH-treated conspecifics (Yi et al., 2009).

This study has shown that volatile emission in *T. vaporariorum*-infested plants is a dynamic process that involves a time-dependent variation in the VOC composition. This includes changes in the levels of certain VOCs that could act as biologically active compounds, such as MeSA, which when converted to SA *in planta* via specific esterases could orchestrate an effective resistance response. Such a scenario implies that the resistance observed was the result of the accumulation of defensive proteins. This aspect will be more clearly defined when the defensive roles of the proteins encoded by the above marker genes are proven.

Why, however, should volatiles emitted by insect-infested plants be able to confer cross-kingdom resistance against bacteria? It is well-known that the interactions among phyla in nature can be antagonistic, synergistic, or neutral, and at times be difficult to determine. This degree of complexity can be found in, for instance, below-ground-above-ground interactions (recently reviewed by van Dam and Heil, 2011; Soler et al., 2012). An increased cross kingdom resistance produced by VOCs that usually act as risk indicators of an impending herbivore infestation, could be a highly desirable adaptive trait in environments where tomato plants are subjected to a high pressure by both insect herbivores and pathogens. However, this requires that tomato's predecessors inhabited environments where herbivore and pathogen attacks were predictable and highly correlated, which is unknown at present. A more plausible explanation is that this trait arose as the result of natural selection for a generalized defensive response effective against a wide range of stressors. This proposal is supported by recent data showing that tomato plants share a SA-based defense strategy against whiteflies, aphids, and biotrophic bacteria, which can manipulate jasmonate-based signaling to antagonize SA responses and gain access to their hosts. Hence, plants have evolved complex and interconnected signaling pathways to cope with stress. Jasmonates themselves represent an apt example of this complexity, since they not only regulate resistance against several insect species and microbial pathogens, but also responses to UV radiation, ozone, salinity, drought, and several other abiotic stresses (Browse and Howe, 2008).

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References

AMENT, K., KANT, M. R., SABELIS, M. W., HARING, M. A., and SCHUURINK, R. C. 2004. Jasmonic acid is a key regulator of spider mite-induced volatile terpenoid and methyl salicylate emission in tomato. *Plant Physiol.* 135:2025–2037.

AOKI, K., YANO, K., SUZUKI, A., KAWAMURA, S., SAKURAI, N., SUDA, K., KURABAYASHI, A., SUZUKI, T., TSUGANE, T., WATANABE, M.,

Ooga, K., Torii, M., Narita, T., Shini, T., Kohara, Y., Yamamoto, N., Takahashi, H., Watanabe, Y., Egusa, M., Kodama, M., Ichinose, Y., Kikuchi, M., Fukushima, S., Okabe, A., Arie, T., Sato, Y., Yazawa, K., Satoh, S., Omura, T., Ezura, H., and Shibata, D. 2010. Large-scale analysis of full-length cDNAs from the tomato (*Solanum lycopersicum*) cultivar Micro-Tom, a reference system for the Solanaceae genomics. *BMC Genomics* 11:210.

Arimura, G., Shiojiri, K., and Karban, R. 2010. Acquired immunity to herbivory and allelopathy caused by airborne plant emissions. *Phytochemistry* 71:1642–1649.

Arimura, G., Ozawa, R., and Maffei, M. E. 2011. Recent advances in plant early signaling in response to herbivory. *Int. J. Mol. Sci.* 12:3723–3739.

Attaran, E., Zeier, T. E., Griebel, T., and Zeier, J. 2009. Methyl salicylate production and jasmonate signaling are not essential for systemic acquired resistance in Arabidopsis. *Plant Cell* 21:954–971.

Birkett, M. A., Chamberlain, K., Guerrieri, E., Pickett, J. A., Wadhams, L. J., and Yasuda, T. 2003. Volatiles from whitefly-infested plants elicit a host-locating response in the parasitoid, *Encarsia Formosa*. *J. Chem. Ecol.* 29:1589–1600.

Berlinger, M. J. 1986. Host plant resistance to *Bemisia tabaci*. *Agric. Ecosyst. Environ.* 17:69–82.

Blande, J. D., Korjus, M., and Holopainen, J. K. 2010. Foliar methyl salicylate emissions indicate prolonged aphid infestation on silver birch and black alder. *Tree Physiol.* 30:404–416.

Bleeker, P. M., Diergaarde, P. J., Ament, K., Guerra, J., Weidner, M., Schütz, S., De Both, M. T. J., Haring, M. A., and Schuurink, R. C. 2009. The role of specific tomato volatiles in tomato-whitefly interaction. *Plant Physiol.* 151:925–935.

Browse, J. and Howe, G. A. 2008. New weapons and a rapid response against insect attack. *Plant Physiol.* 146:832–838.

Buttery, R. G., Ling, L. C., and Light, D. M. 1997. Tomato leaf volatile aroma components. *J. Agric. Food Chem.* 35:1039–1042.

Byrne, D. N. and Miller, W. B. 1990. Carbohydrate and amino acid composition of phloem sap and honeydew produced by *Bemisia tabaci*. *J. Insect Physiol.* 36:433–439.

Choh, Y., Shimoda, T., Ozawa, R., Dicke, M., and Takabayashi, J. 2004. Exposure of lima bean leaves to volatiles from herbivore-induced conspecific plants results in emission of carnivore attractants: active or passive process? *J. Chem. Ecol.* 30:1305–1317.

Chou, Y., Polansky, A. M., and Mason, R. L. 1998. Transforming non-normal data to normality in statistical process control. *J. Qual. Technol.* 30:133–141.

Colby, S. M., Crock, J., Dowdle-Rizzo, B., Lemaux, P. G., and Croteau, R. 1998. Germacrene C synthase from *Lycopersicon esculentum* cv. VFNT Cherry tomato: cDNA isolation, characterization, and bacterial expression of the multiple product sesquiterpene cyclase. *Proc. Natl. Acad. Sci. U. S. A.* 95:2216–2221.

Corrado, G., Sasso, R., Pasquariello, M., Iodice, L., Carretta, A., Cascone, P., Ariati, L., Digilio, M. C., Guerrieri, E., and Rao, R. 2007. Systemin regulates both systemic and volatile signaling in tomato plants. *J. Chem. Ecol.* 33:669–681.

van Dam, N. M. and Heil, M. 2011. Multitrophic interactions below and above ground: en route to the next level. *J. Ecol.* 99:77–88.

Estrada-hernández, M. G., Valenzuela-soto, J. H., Ibarra-laclette, E., and Délano-frier, J. P. 2009. Differential gene expression in whitefly *Bemisia tabaci*-infested tomato (*Solanum lycopersicum*) plants at progressing developmental stages of the insect's life cycle. *Physiol. Plant.* 137:44–60.

Expósito-Rodríguez, M., Borges, A. A., Borges-Pérez, A., and Pérez, J. A. 2008. Selection of internal control genes for quantitative real-time RT-PCR studies during tomato development process. *BMC Plant Biol.* 8:131.

Frost, C. J., Mescher, M. C., Carlson, J. E., and de Moraes, C. M. 2008. Plant defense priming against herbivores: getting ready for a different battle. *Plant Physiol.* 146:818–824.

Goellner, K. and Conrath, U. 2008. Priming: it's all the world to induced disease resistance. *Eur. J. Plant Pathol.* 121:233–242.

Heil, M. and Adame-Álvarez, R. M. 2010. Short signalling distances make plant communication a soliloquy. *Biol. Lett.* 6:843–845.

Heil, M. and Karban, R. 2010. Explaining evolution of plant communication by airborne signals. *Trends Ecol. Evol.* 25:137–144.

Hu, Z., Shen, Y., and Su, X. 2009. Saturated aldehydes C6–C10 emitted from ashleaf maple (*Acer negundo* L.) leaves at different levels of light intensity, O_2, and CO_2. *J. Plant Biol.* 52:289–29.

Inbar, M. and Gerling, D. 2008. Plant-mediated interactions between whiteflies, herbivores, and natural enemies. *Annu. Rev. Entomol.* 53:431–448.

Isaacs, R., Willis, M. A., and Byrne, D. N. 1999. Modulation of whitefly take-off and flight orientation by wind speed and visual cues. *Physiol. Entomol.* 24:311–318.

James, D. G. 2003. Field evaluation of herbivore-induced plant volatiles as attractants for beneficial insects: methyl salicylate and the green lacewing, *Chrysopa nigricornis*. *J. Chem. Ecol.* 29:1601–1609.

Jansen, R. M. C., Miebach, M., Kleist, E., van Henten, E. J., and Wildt, J. 2009. Release of lipoxygenase products and monoterpenes by tomato plants as an indicator of *Botrytis cinerea*-induced stress. *Plant Biol.* 11:859–868.

Jones, D. R. 2003. Plant viruses transmitted by whiteflies. *Eur. J. Plant Pathol.* 109:195–219.

Kant, M. R., Ament, K., Sabelis, M. W., Haring, M. A., and Schuurink, R. C. 2004. Differential timing of spider mite-induced direct and indirect defenses in tomato plants. *Plant Physiol.* 135:483–495.

Kishimoto, K., Matsui, K., Ozawa, R., and Takabayashi, J. 2005. Volatile C6-aldehydes and allo-ocimene activate defense genes and induce resistance against *Botrytis cinerea* in *Arabidopsis thaliana*. *Plant Cell Physiol.* 46:1093–1102.

Lee, J. C. 2010. Effect of methyl salicylate-based lures on beneficial and pest arthropods in strawberry. *Environ. Entomol.* 39:653–660.

Liu, P. P., von Dahl, C. C., Park, S. W., and Klessig, D. F. 2011. Interconnection between methyl salicylate and lipid-based long-distance signaling during the development of systemic acquired resistance in Arabidopsis and tobacco. *Plant Physiol.* 155:1762–1768.

Livak, K. J. and Schmittgen, T. D. 2001. Analysis of relative gene expression data using real- time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. *Methods* 25:402–408.

Manosalva, P. M., Park, S. W., Forouhar, F., Tong, L., Fry, W. E., and Klessig, D. F. 2010. *Methyl esterase 1* (*StMES1*) is required for systemic acquired resistance in potato. *Mol. Plant Microbe Interact.* 23:1151–1163.

Martin, J. H. 1987. An identification guide to common whiteflies pest species of the world (Homoptera: Aleyrodidae). *Trop. Pest Man.* 33:298–322.

McCollum, T. G., Stoffell, P. J., Powell, C. A., Cantliffe, D. J., and Hanif-Khan, S. 2004. Effects of silverleaf whitefly feeding on tomato fruit ripening. *Postharvest Biol. Technol.* 31:183–190.

Park, S. W., Liu, P. P., Forouhar, F., Vlot, A. C., Tong, L., Tietjen, K., and Klessig, D. F. 2009. Use of a synthetic salicylic acid analog to investigate the roles of methyl salicylate and its esterases in plant disease resistance. *J. Biol. Chem.* 284:7307–7317.

Puthoff, D. P., Holzer, F. M., Perring, T. M., and Walling, L. L. 2010. Tomato pathogenesis-related protein genes are expressed in

response to *Trialeurodes vaporariorum* and *Bemisia tabaci* biotype B feeding. *J. Chem. Ecol.* 36:1271–1285.

RAMADAN, A., MUROI, A., and ARIMURA, G. 2011. Herbivore-induced maize volatiles serve as priming cues for resistance against post-attack by the specialist armyworm *Mythimna separate*. *J. Plant Interact.* 6:155–158.

RODRÍGUEZ-SAONA, C., CRAFTS-BRANDNER, S. J., and CAÑAS, L. A. 2003. Volatile emissions triggered by multiple herbivore damage: beet armyworm and whitefly feeding on cotton plants. *J. Chem. Ecol.* 29:2539–2550.

RYU, C. M., FARAG, M. A., HU, C. H., REDDY, M. S., KLOEPPER, J. W., and PARÉ, P. W. 2004. Bacterial volatiles induce systemic resistance in Arabidopsis. *Plant Physiol.* 134:1017–1026.

SAKIA, R. M. 1992. The Box-Cox transformation technique: a review. *Statistician* 41:169–178.

SÁNCHEZ-HERNÁNDEZ, C., LÓPEZ, M. G., and DÉLANO-FRIER, J. P. 2006. Reduced levels of volatile emissions in jasmonate-deficient *spr2* tomato mutants favour oviposition by insect herbivores. *Plant Cell Environ.* 29:546–557.

SHULAEV, V., SILVERMAN, P., and RASKIN, I. 1997. Airborne signalling by methyl salicylate in plant pathogen resistance. *Nature* 385:718–721.

SNOEREN, T. A. L., MUMM, R., POELMAN, E. H., YANG, Y., PICHERSKY, E., and DICKE, M. 2010. The herbivore-induced plant volatile methyl salicylate negatively affects attraction of the parasitoid *Diadegma semiclausum*. *J. Chem. Ecol.* 36:479–489.

SOLER, R., VAN DER PUTTEN, W. H., HARVEY, J. A., VET, E. M., DICKE, M., and BEZEMER, T. M. 2012. Root herbivore effects on aboveground multitrophic interactions: patterns, processes and mechanisms. *J. Chem. Ecol.* 38:755–767.

SONG, W., MA, X., TAN, H., and ZHOU, J. 2011. Abscisic acid enhances resistance to *Alternaria solani* in tomato seedlings. *Plant Physiol. Biochem.* 49:693–700.

STANSLY, P. A. and MCKENZIE, C. L. 2007. Fourth International *Bemisia* Workshop International Whitefly Genomics Workshop. *J. Insect Sci.* 8:4.

TANAKA, K. and KOMATSU, K. 2008. Comparative study on volatile components of *Nardostachys* rhizome. *J. Nat. Med.* 62:112–116.

TRONCOSO, A. J., CABEZAS, N. J., FAÚNDEZ, E. H., URZÚA, A., and NIEMEYER, H. M. 2010. Host-mediated volatile polymorphism in a parasitic plant influences its attractiveness to pollinators. *Oecologia* 162:413–425.

VALENZUELA-SOTO, J. H., IRUEGAS-BOCARDO, F., MARTIÍNEZ-GALLARDO, N. A., MOLINA-TORRES, J., GÓMEZ-LIM, M. A., and DÉLANO-FRIER, J. P. 2011. Transformed tobacco (*Nicotiana tabacum*) plants over-expressing a peroxisome proliferator-activated receptor gene from *Xenopus laevis* (xPPARa) show increased susceptibility to infection by virulent *Pseudomonas syringae* pathogens. *Planta* 233:507–521.

VLOT, A. C., LIU, P. P., CAMERON, R. K., PARK, S. W., YANG, Y., KUMAR, D., ZHOU, F., PADUKKAVIDANA, T., GUSTAFSSON, C., PICHERSKY, E., and KLESSIG, D. F. 2008. Identification of likely orthologs of tobacco salicylic acid-binding protein 2 and their role in systemic resistance in *Arabidopsis thaliana*. *Plant J.* 56:445–456.

WALLING, L. L. 2008. Avoiding effective defenses: strategies employed by phloem-feeding insects. *Plant Physiol.* 146:859–866.

YI, H. S., HEIL, M., ADAME-ÁLVAREZ, R. M., BALLHORN, D., and RYU, C. M. 2009. Airborne induction and priming of plant resistance to a bacterial pathogen. *Plant Physiol.* 151:2152–2161.

YI, H. S., RYU, C. M., and HEIL, M. 2010. Sweet smells prepare plants for future stress. Airborne induction of plant disease immunity. *Plant Signal. Behav.* 5:528–531.

ZHANG, P. J., ZHENG, S. J., VAN LOON, J. J. A., BOLAND, W., DAVID, A., MUMM, R., and DICKE, M. 2009. Whiteflies interfere with indirect plant defense against spider mites in lima bean. *Proc. Natl. Acad. Sci. U. S. A.* 106:21202–21207.