Better Together: Volatile-mediated Interguild Effects on the Preference of Tuta Absoluta and Trialeurodes Vaporariorum for Tomato Plants

Federico RODRIGO
Laboratorio de Ecología Química, Facultad de Química, Universidad de la República
Anna P. BURGUEÑO
Laboratorio de Ecología Química, Facultad de Química, Universidad de la República
Andrés González
Laboratorio de Ecología Química, Facultad de Química, Universidad de la República
Carmen ROSSINI (crossini@fq.edu.uy)
Laboratorio de Ecología Química, Facultad de Química, Universidad de la República

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Abstract

Plant-herbivore interactions have been extensively studied in tomato plants and their most common pests. Tomato plant chemical defenses, both constitutive and inducible, play a role in mediating these interactions. Damaged tomato plants alter their volatile profiles, affecting herbivore preferences between undamaged and damaged plants. However, previous studies on tomato volatiles and herbivore preferences have yielded conflicting results, both in the volatile chemistry itself as well as in the attraction/repellent herbivore response. This study revisits the volatile-mediated interactions between tomato plants and two of their main herbivores: the leafminer *Tuta absoluta* and the whitefly *Trialeurodes vaporariorum*. Tomato plant volatiles were analyzed before and after damage by each of these herbivores, and the preference for oviposition (*T. absoluta*) and settling (*T. vaporariorum*) on undamaged and damaged plants was assessed both after conspecific and heterospecific damage. We found that both insects consistently preferred damaged plants over undamaged plants. The emission of herbivore-induced plant volatiles (HIPVs) increased after *T. absoluta* damage but decreased after *T. vaporariorum* damage. While some of our findings are in line with previous reports, *T. absoluta* preferred to oviposit on plants damaged by conspecifics, which differs from earlier studies. A comparison of HIPVs emitted after damage by *T. absoluta* and *T. vaporariorum* revealed differences in up- or down-regulation, as well as significant variations in specific compounds (12 for *T. absoluta* and 26 for *T. vaporariorum* damaged-plants). Only two compounds, *E*-caryophyllene and tetradecane, significantly varied because of damage by either herbivore, in line with the overall variation of the HIPV blend. Differences in HIPVs and herbivore preferences may be attributed to the distinct feeding habits of both herbivores, which activate different defensive pathways in plants. The plant's challenge in simultaneously activating both defensive pathways may explain the preference for heterospecific damaged plants found in this study, which are also in line with our own observations in greenhouses.

INTRODUCTION

Insect selection of host plants to settle, feed or oviposit is affected by many factors; among others, previous insects experience (including habituation or sensitization (Heard 1999)), plant quality for self or the progeny (Awmack and Leather 2002), the presence of plant viruses (Chen et al. 2017; Mann et al. 2009) or beneficial plant-associated microorganisms (Grunseich et al. 2019). Insects also choose their host plants based on defenses that plants possess or may produce (Rodriguez-Lopez et al. 2020) due to previous herbivore infestation levels, either by conspecifics (Zhang et al. 2014) or by heterospecific herbivores (Saad et al. 2015). Numerous studies have focused on how herbivorous insects use chemical cues to select their host plants (preference). Such cues include volatile organic compounds (VOCs) detected remotely through the olfactory system, as well as non-volatiles detected after direct contact with the plant (Schoonhoven et al. 2005). In the case of the tomato plants (*Solanum lycopersicum*, Solanaceae) the presence of trichomes and other secondary metabolites (e.g., acyl sugars, alkaloids, methyl ketones) also play a role in host selection by the tomato herbivores (Oliveira et al. 2012; Regina Gontijo Labory C 1999; Sohrabi et al. 2016; Tian et al. 2012).

Volatile organic compounds are secondary metabolites released by plants that mediate important ecological processes, including interactions between plants and their herbivores (Furstenberg-Hagg et al. 2013), their pollinators (Raguso 2004), beneficial natural enemies such as predators and parasitoids (Dicke 2015), and even other plants in the surroundings (Baldwin et al. 2006). VOCs are differentially produced according both to environmental factors that affect plant development (Beck et al. 2014) and to biotic stress affecting the plant (Lucas-Barbosa 2016; Venkatesan 2015). Herbivore damage induces changes in the plant's emitted volatiles, so called herbivore-induced plant volatiles (HIPVs), which may modulate plant-herbivore interactions and mediate attraction of herbivore natural enemies (Ayelo et al. 2021c). These HIPVs function as indirect defenses and are modulated mainly by the activation of the jasmonic acid (JA) and salicylic acid (SA) pathways. Cross-talking between pathways regulates and balances the final outcome of the plant's induced response (Glas 2014). These pathways are differentially activated depending on the insect feeding habits: while chewer insects activate the JA pathway; phloem feeders activate mainly the SA pathway (Glas 2014; Lin et al. 2019; Pieterse et al. 2012). Therefore, plants modulate their induced defensive response to different herbivores because the JA and SA defense pathways usually

exhibit negative cross-talk; that is, the upregulation response produced by one of the hormones lowers the response regulated by the other one (Pieterse et al. 2012). Herbivores, in turn, are able to manipulate these plant defenses (Pieterse et al. 2012).

In the case of tomato plants, HIPVs play a role in their interactions with different herbivore guilds. These include the phloem-feeder whiteflies, such as *Bemisia tabaci* and *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) (Lorenzo et al. 2016; Rodriguez-Lopez et al. 2020), as well as leaf chewers of the order Lepidoptera (Tian et al. 2014), including *Tuta absoluta* (Lepidoptera: Gelechiidae) (Anastasaki et al. 2018), and Coleoptera (Tian et al. 2012). *Tuta absoluta* specializes in Solanaceae and particularly in tomato plants as its main host (Silva et al. 2021b). Their larvae are chewing miners that feed in the leaf mesophyll; they produce galleries through which they move to other parts of the plant such as the fruits (Gontijo et al. 2013), causing their decay (Bentancourt 1995; Da Silva Galdino et al. 2015). While some evidence on leaf mesophyll feeding has been reported for adults (Baetan et al. 2015), they feed mostly on nectar, without causing significant damage to the plant. When choosing their host plant, *T. absoluta* females respond preferentially to tomato volatiles over potato volatiles (Caparros Megido et al. 2014) and oviposit at higher rates in tomato than in potato (Caparros Megido et al. 2014; Sridhar et al. 2015) or eggplants (Sridhar et al. 2015). Female ability to discriminate among plant volatiles was also observed when given the option between non-damaged and damaged (by conspecific larvae) tomato plants, preferring to oviposit on undamaged plants (Anastasaki et al. 2018; Bawin et al. 2014; Maneesha et al. 2021). *Trialeurodes vaporariorum and B. tabaci* are polyphagous and cosmopolitan leaf sucker pests that cause direct and indirect damage to plants both as nymphs and as adults (Rodríguez 2003). Feeding and oviposition preferences differ between both whitefly species: while *T. vaporariorum* prefers to settle and oviposit on tomato over pepper plants, *B. tabaci* prefers to settle on pepper but lays more eggs on tomato plants (Lorenzo et al. 2016). *Bemisia tabaci* is attracted to, prefers to settle and oviposit, and performs better, on conspecific-damaged tomato plants rather than on undamaged plants (Su et al. 2018). *Trialeurodes vaporariorum* males are more attracted to conspecific-damaged plant volatiles, while females are attracted to volatiles from undamaged plants. These attraction responses and preferences seem to be guided by plant volatiles, since volatiles produced by the insects themselves are not attractive to conspecific females or males (Darshanee et al. 2017).

While several studies have focused on insect-plant interactions involving whiteflies and *Tuta absoluta*, only a few have **studied these interactions when other herbivores are involved**. It is known that previous herbivore attack change other herbivore preferences for settling, feeding, ovipositing, or even their performance (Karban 1989). For instance, *B. tabaci* prefers to settle and lay eggs on cucumber plants previously infested by *Tetranychus cinnabarinus* (Acari: Tetranychidae), but not on plants pre-infested by *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae) (Lin et al. 2019) or by *Myzus persicae* (Hemiptera: Aphididae) (Tan et al. 2014). In the latter case, *B. tabaci* preference for undamaged vs. damaged plants was correlated with the emission of HIPVs by tomato (Tan and Liu 2014). The differential preference of *B. tabaci* towards damaged plants may arise from the different feeding habits of the herbivores, which activate different defensive pathways in the plants (Lin et al. 2019). In the case of *Trichoplusia ni* (Lepidoptera: Noctuidae) oviposition preferences changed depending on which herbivore has previously damaged the plants. It prefers ovipositing on undamaged soy plants rather than on *Spodoptera frugiperda* (Lepidotera: Noctuidae) or *B. tabaci*-damaged plants. However, *T. ni* prefers laying eggs on soy plants where there are heterospecific eggs rather than on undamaged ones (Coapio et al. 2016). In the case of *T. absoluta*, mated females were more attracted to volatiles from, and preferred laying eggs on undamaged plants rather than on plants damaged by *Liriomyza trifolii* (Diptera: Agromyzidae) (Maneesha et al. 2021). Finally, *T. absoluta* larva feeding on the same leaf as *B. tabaci* nymphs showed a decreased performance; but the performance of *B. tabaci* nymphs was not affected by previous feeding of *T. absoluta* larvae (Mouttet et al. 2013).

We have observed both insects usually co-occurring on the same plants in greenhouse crops (unpublished), so we set up to study this interguild herbivore-plant system in more detail. Specifically, this study focuses first on the production of volatiles by tomato plants infested by two of the main insect pests in South America: the tomato moth *T. absoluta* and the whitefly *T. vaporariorum*. Second, we aimed to evaluate how pre-infestation of *T. absoluta* or *T. vaporariorum* affects their oviposition and settlement preferences. Understanding these intraguild interactions between herbivore insects that are relevant as tomato pests may provide valuable inputs for tomato production.

METHODS AND MATERIALS

Plants and Insects. All plants and insects were reared under the same controlled conditions (25°C, 16:8 L:D, r.H. = 70%). Tomato plants, *S. lycopersicum* cv. San Pedro, were grown in individual pots (12 cm h x 12 cm diam.) and watered either three times a week or as needed according to the plant water demand. *Tuta absoluta* were reared in laboratory cages (15 x 15 x 30 cm, 6.7 L) covered by voile and fed with potted fully-grown tomato plants with at least 7 leaves with 7 leaflets each. The plants were about 1 month old and were replaced twice a week. The adults were separated from the larvae every 3 days. *Trialeurodes vaporariorum* were weekly collected as adults from pesticide-free tomato crops grown in greenhouses, then kept in the laboratory on potted tomato plants like those described for *T. absoluta*.

Damaged Plants. Damage procedures were done under the same controlled laboratory conditions mentioned above. Damage was performed for both insects on fully grown plants confined in cages (55 cm, 166 L) by exposing the plants to the insects for 24 h. The plants were then used either for bioassays or for headspace collection of plant volatiles. In the case of *T. absoluta*-damaged plants, preliminary tests were performed with different ratio of larvae/leaflet to obtain plants with at least 25% of the leaflet surface damaged after 24 h, but without dying during the assay time (72 h). The ratio of 1 larva (L3) for every two leaflets (that is between 20 to 25 larvae per plant) was chosen. Leafminer larvae were not removed from the plants after the 24-h initial damage to cause continuous damage throughout the experiments, and to avoid causing mechanical damage to the plant, which might modify plant volatile emissions (Raghava et al. 2010). At the end of the experiments leaf damage was visually evaluated resulting in ca. 33% leaf surface damage. In the case of *T. vaporariorum*-damaged plants, 75 field-collected adults were placed on fully grown- tomato plants for 24 h. As these insects were not sexed in advance, damage to the plants could have been caused by both feeding and oviposition. As with leafminer larvae, adults were not removed from the plant after the initial 24-h damage period, to have continuous damage on the plant. Therefore, when performing settling preference assays with *T. vaporariorum*, the number of settled adults was corrected as explained below.

Preference Bioassays. All bioassays were run as choice experiments in which one intact and one damaged plant of the same age and foliar development were offered in opposite sides of a cage (55 x 55 x 120 cm, 363 L). To assess oviposition preference by *T. absoluta*, 50 adults of both sexes were released. After 72 h, the number of eggs laid on each plant was registered (n = 12 for *T. absoluta*-damaged vs. undamaged plants; n = 7 for *T. vaporariorum*-damaged vs. undamaged plants). For assessing *T. vaporariorum* settlement preferences, 75 adults were released and the number of whiteflies settled on each plant was registered at the end of the assay (72 h) for undamaged vs. *T. absoluta*-damaged plants (n = 11), or after 24, 48 and 72 h for undamaged vs. *T. vaporariorum*-damaged plants (n = 11).

To account for the previous presence of *T. vaporariorum* in the damaged plants, we ran preliminary tests that showed that no more than 2.5% of whiteflies migrate from the damaged to the healthy plant in the 72-h assay time window. Besides, the percentage of whitefly death during 72 h was 30% in the first day, 14.4% in the second and 13.7% in the third (n = 15). According to these preliminary results, when counting settled whiteflies in conspecific-damaged plants, corrections were made by subtracting the whiteflies initially used for damaging the plants; that is, 52 (30% of 75) individuals in day one, 45 (14.4% of 52) in in day two, and 39 (13.7% of 45) in day three. Both preference bioassays and volatile collection in the case of *T. absoluta*-damage were conducted in January-March 2019; and in January-March 2021 for *T. vaporariorum's.*

Plant Volatile Chemistry. To obtain volatile extracts from the same plants before and after damage, volatile collections were first performed from undamaged plants for 72 h, then the same plants were damaged as described previously, and another volatile collection (72 h) was performed from the damaged plants. This collection-damage-collection procedure was performed five times in blocks of 3 plants, reaching 15 plant volatile extracts for both *T. absoluta*- and *T. vaporariorum*-damaged plants. Two plants and their volatile collections had to be discarded from each group, therefore reaching the final sample sizes as 13 plant volatile extracts sampled before and after damage by each herbivore.

Volatile collections were done by enclosing potted tomato plants, with their pots wrapped in aluminum foil, in Teflon-sealed glass cylinders (37 cm h x 28 cm d; 17 L) at room temperature. A background volatile control was done in parallel for each

group of three sampled plants, using a pot with soil wrapped with aluminum foil. A stream of charcoal-filtered air was pushed through the system at 2 L/min using an electric air compressor (Toshiba TOSCON) and simultaneously pulled from the plant chamber at a flow of 1 L/min for 72 h, using a CASELLA Apex 2 pump. Volatiles were adsorbed on 50 mg HayeSep Q (Hayes Separations, Inc.), then eluted with 1 mL of double-distilled hexane and added with tridecane (24 µg) as internal standard (IS). After elution, the solution was concentrated to 100 µL under a N_2 stream.

A Shimadzu QP2010 plus gas chromatograph coupled to mass spectrometer (GC-MS) was used for volatiles characterization. The analyses were performed with an OPTIMA-5-MS column (30 m x 0.25 mm id x 0.25-µm film thickness; Macherey-Nagel). The analytical conditions were as follows: gas carrier: He (1 mL/min); oven temperature: from 40°C (1 min) to 160°C (3°C/min), 235°C (5°C/min) and finally to 280°C (20°C/min, 2 min); injector and detector temperatures: 250°C; injection 1 µL in the splitless mode; ionization potential: 70 eV; scan range: 40–350 m/z. The identification of volatiles was performed by comparing calculated Retention Indices (RI) with those reported by Adams (2007) and by comparison of fragmentation patterns with those contained in NIST 05 (Linstrom and Mallard 2005) and Adams (2007) mass spectrum libraries. The amount of each volatile was quantified by manually integration and expressed as µg of internal standard.

Statistical Analyses. In bioassays, the number of eggs laid by *T. absoluta* and the corrected number of *T. vaporariorum* adults settled in undamaged vs. damaged plants were compared with Wilcoxon tests, using the VassarStats website (Lowry 2023). Volatile profiles of damaged and undamaged plants were analyzed using the online Metaboanalyst platform (Chong et al. 2018). The GC-MS data were not filtered before multivariate analyses. Normalization, scaling, and centering of the data was done (operations used are indicated in each result) (Chong et al. 2018). Outliers were not detected. GC-MS profiles of volatiles were first explored with unbiased Principal Component Analyses (PCA). Further analyses for identifying peaks that contribute to the differentiation of samples were done using Partial Least Square-Discriminant Analyses (PLS-DA). In these supervised models, the health status (2 levels: damaged and undamaged plants) was included as the classification variable in the model. The PLS-DA models were cross validated with permutation tests (number of permutations as indicated in each result). Then, the PLS loading and variable influence on the projection (VIP) scores were used to make a selection of peaks of interest (VIP > 1) (Xia and Wishart 2016) that contributed to the differentiation of undamaged vs. *T. absoluta*-damaged plants and between undamaged and *T. vaporariorum*-damaged plants. Random Forest Analyses were also run. In this case, during tree construction for the classification process, about one-third of the instances were left out of the bootstrap sample. The out-of-bag (OOB) error was calculated and used for variable importance estimation; and precision (percent of correct predictions), recall (percent of correct classification); and Prior Probability (percent expected by chance) were calculated. Besides, data on individual compounds were also subjected to conventional univariate analyses (t-tests on paired samples).

RESULTS

Preference Bioassays with T. absoluta-damaged Plants. *Tuta absoluta* females preferred to lay eggs on conspecific-damaged plants (41 ± 5 total number of eggs/plant) rather than on undamaged plants (22 ± 3 total number of eggs/plant; p = 0.005, Wilcoxon test, Fig. 1A). *Trialeurodes vaporariorum* also preferred to settle on *T. absoluta*-damaged plants (20 ± 5 whiteflies/plant) rather than on undamaged plants (9 ± 1 whiteflies/plant; p = 0.03, Wilcoxon test, Fig. 1B).

Insert Fig. 1 *here*

Preference bioassays on T. vaporariorum-damaged plants. *Tuta absoluta* preferred laying eggs on *T. vaporariorum*-damaged (42 ± 5 total number of eggs/plant) rather than on undamaged plants (20 ± 4) (p = 0.03, Wilcoxon, Fig. 2A). For conspecific-damaged vs. undamaged plants, *T. vaporariorum* preferred settling on damaged plants in all of the three days assessed: day one (44 ± 5) vs. (17 ± 2), day two (46 ± 6) vs. (16 ± 3) and day three (38 ± 6) vs. (13 ± 2) (p = 0.006, p = 0.001, p = 0.021, respectively, Wilcoxon tests, Fig. 2B).

Insert Fig. 2 *here*

Plant Volatile Chemistry. The volatile profiles emitted by tomato plants were complex as expected (Fig. S1). Combining the volatiles from the four plant treatments studied, 147 chromatographic peaks were detected (Tables 1 and 2). Among these peaks, 103 were from plants damaged by *T. absoluta* and their corresponding undamaged plants, and 92 were from plants damaged by *T. vaporariorum* and their corresponding undamaged plants (Table 3). While the tomato cultivar was the same for all experiments (San Pedro), volatiles from undamaged plants used for *T. absoluta* damage experiments differed from the volatiles from undamaged plants used for *T. vaporariorum* damage (Tables 1 and 2 and Fig. S1). These differences probably arise from the 1-year difference in the time in which the assays were performed for each herbivore. Therefore, volatiles from *T. absoluta* damaged plants and from *T. vaporariorum* damaged plants will be analyzed separately. While only 49 out of the 147 peaks detected were present in all plants in the different treatments, these common compounds accounted for most of the volatiles (average range from 73 to 78% among the four treatments), and most of them have been reported in previous studies (Anastasaki et al. 2018; Ángeles López et al. 2012; Ayelo et al. 2021a; Ayelo et al. 2021c; Caparros Megido et al. 2014; Milonas et al. 2019; Proffit et al. 2011; Silva et al. 2018; Silva et al. 2017). Identified compounds belong to the common classes usually found in plant volatiles (Table 3).

Table 1

Volatiles emitted by undamaged and *T. absoluta*-damaged tomato plants (N = 13 pairs of undamaged/damaged plants).

n	Compound	Class[a]	ARi_{rep}[b]	ARi_{calc}[c]	Undamaged plants[d]	*T. absoluta*-damaged plants[d]	t test (p value)
1	octane	HC	800	800	0.09 ± 0.09	0.3 ± 0.2	
2	unk* 1	NI	-	870	0.03 ± 0.02	0.003 ± 0.002	
3	unk 2	NI	-	872	0.013 ± 0.009	0.003 ± 0.003	
4	unk 3	NI	-	884	0.02 ± 0.01	0 ± 0	
5	nonane	HC	900	885	0.03 ± 0.01	0.06 ± 0.027	
6	α-pinene	MT	932	921	0.3 ± 0.1	0.16 ± 0.05	
7	3,7,7-trimethyl-1,3,5-cycloheptatriene	MT	971	962	0.5 ± 0.2	0.4 ± 0.1	
8	sabinene	MT	969	966	0.08 ± 0.03	0.04 ± 0.01	
9	β-pinene	MT	974	969	0.06 ± 0.03	0.05 ± 0.02	
10	1-octen-3-ol	OI	974	975	0.03 ± 0.01	0.08 ± 0.02	
11	2,2,4,6,6-pentamethyl-heptane	HC	991	985	0.06 ± 0.02	0.013 ± 0.004	
12	β-myrcene	MT	988	989	0.6 ± 0.2	0.5 ± 0.1	
13	butyl butanoate	Ester	993	996	0.06 ± 0.03	0.012 ± 0.006	
14	δ2-carene	MT	1001	999	2.0 ± 0.5	1.9 ± 0.4	
15	α-phellandrene	MT	1002	1002	0.8 ± 0.3	0.6 ± 0.2	
16	3-carene	MT	1011	1007	0.022 ± 0.004	0.11 ± 0.04	
17	α-terpinene	MT	1014	1014	0.23 ± 0.09	0.23 ± 0.08	
18	p-cymene	MT	1020	1022	0.23 ± 0.07	0.35 ± 0.07	
19	β-phellandrene	MT	1025	1026	7 ± 2	7 ± 2	
20	2-ethyl-1-hexanol	OI	1030	1028	1 ± 1	0.2 ± 0.1	
21	3,7-dimethyl-nonane	HC	1038	1033	0.07 ± 0.04	0.02 ± 0.01	
22	(*Z*)β-ocimene	MT	1032	1037	0.07 ± 0.02	0.08 ± 0.02	
23	(*E*)β-ocimene	MT	1044	1047	0.24 ± 0.08	0.4 ± 0.1	
24	γ-terpinene	MT	1054	1056	0.09 ± 0.04	0.09 ± 0.03	
25	terpinolene	MT	1086	1086	0.13 ± 0.06	0.15 ± 0.04	
26	n-undecane	HC	1100	1098	0.04 ± 0.04	0.3 ± 0.1	0.005
27	n-nonanal	Ald	1100	1102	0.01 ± 0.01	0.13 ± 0.08	
28	phenyl ethyl alcohol	Arom	1106	1112	0.10 ± 0.03	0.11 ± 0.03	
29	menthone	Mt ox	1148	1148	0.040 ± 0.002	0.040 ± 0.005	

n	Compound	Class[a]	ARi_{rep}[b]	ARi_{calc}[c]	Undamaged plants[d]	*T. absoluta*-damaged plants[d]	t test (p value)
30	dill ether	Mt ox	1184	1183	0.03 ± 0.02	0.04 ± 0.01	
31	3*Z*-hexenyl butanoate	Ester	1184	1186	0.005 ± 0.002	0.29 ± 0.09	0.002
32	methyl salicylate	MeSa	1190	1191	0.05 ± 0.03	0.18 ± 0.08	
33	dodecane	HC	1200	1198	0.016 ± 0.01	0.02 ± 0.01	
34	n-decanal	Ald	1201	1204	0.008 ± 0.008	0.06 ± 0.05	
35	2*e*-decenal	Ald	1260	1261	0 ± 0	0.07 ± 0.04	0.004
36	2,6,11-trimethyl-dodecane	STm	1275	1280	0.05 ± 0.02	0.011 ± 0.005	
37	1-tridecene	HC-ene	1290	1290	0.004 ± 0.001	0.007 ± 0.005	
38	indole	Indole	1290	1294	0.011 ± 0.007	0.06 ± 0.03	0.005
39	carvacrol	MT	1298	1297	0.01 ± 0.01	0.05 ± 0.01	0.003
40	undecanal	MT	1305	1307	0.06 ± 0.05	0.05 ± 0.03	
41	2*e*,4*e*-decadienol	Ol	1319	1321	0.04 ± 0.02	0.03 ± 0.01	
42	δ-elemene	ST	1335	1336	0.6 ± 0.2	2 ± 1	
43	unk 4	HC	-	1359	0.02 ± 0.01	0.04 ± 0.02	
44	2-methyl-tridecane	HC	1364	1362	0.009 ± 0.007	0.10 ± 0.05	
45	α-copaene	ST	1374	1374	0.07 ± 0.04	0.12 ± 0.05	
46	β-elemene	ST	1389	1389	0.13 ± 0.06	0.3 ± 0.2	
47	tetradecane	HC	1400	1397	0.03 ± 0.03	0.4 ± 0.1	0.005
48	dodecanal	Ald	1408	1407	0.03 ± 0.03	0.3 ± 0.2	
49	*E*-caryophyllene	ST	1417	1416	1.5 ± 0.4	4 ± 1	0.002
50	γ-elemene	ST	1434	1431	0.02 ± 0.01	0.10 ± 0.07	
51	6,9-guaiadiene	ST	1442	1442	0.04 ± 0.02	0.10 ± 0.06	
52	α-humulene	ST	1452	1451	0.3 ± 0.1	1.3 ± 0.6	0.004
53	4-methyl-tetradecane	HC	1469	1461	0.013 ± 0.008	0.15 ± 0.06	
54	3-methyl-tetradecane	HC	1470	1470	0 ± 0	0.022 ± 0.008	
55	4,5-di-epi-aristolochene	ST	1471	1471	0.04 ± 0.03	1.3 ± 0.9	
56	n-dodecanol	Ol	1469	1475	0 ± 0	0.4 ± 0.3	
57	germacrene-d	ST	1484	1478	0.03 ± 0.03	0.16 ± 0.07	

n	Compound	Class[a]	ARi_{rep}[b]	ARi_{calc}[c]	Undamaged plants[d]	*T. absoluta*-damaged plants[d]	t test (p value)
58	(*E*)-β-ionone	Ionone	1487	1486	0.03 ± 0.01	0.3 ± 0.1	< 0.001
59	pentadecane	HC	1500	1495	0.09 ± 0.05	0.10 ± 0.03	
60	ukn HC 1	HC	-	1499	0 ± 0	0.3 ± 0.2	
61	2-hexyl-1-decanol	OI	1504	1507	0.01 ± 0.006	0.14 ± 0.04	< 0.001
62	unk 5	NI	-	1526	0.1 ± 0.05	0.3 ± 0.1	
63	2,6,10-trimethyl-tetradecane	STm	1539	1540	0.06 ± 0.03	0.12 ± 0.06	
64	3,7,11-trimethyl-1,6,10-dodecatrien-3-ol	OI	1564	1559	0.3 ± 0.1	0.7 ± 0.3	
65	3-methyl-pentadecane	HC	1570	1568	0.004 ± 0.003	0.10 ± 0.07	
66	(3*E*,7*E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene	STm	1577	1578	2.6 ± 0.9	5 ± 2	
67	hexadecane	HC	1600	1596	0 ± 0	0.4 ± 0.3	
68	tetradecanal	Ald	1611	1609	0.011 ± 0.008	2 ± 1	
69	benzophenone	Arom	1626	1624	0.003 ± 0.003	0.5 ± 0.5	< 0.001
70	dodecanoic acid 1 methyethyl ester	ester	1618	1626	0.07 ± 0.02	2 ± 2	
71	2-methyl-hexadecane	HC	1666	1666	0.09 ± 0.05	0.29 ± 0.2	
72	3-methyl-hexadecane	HC	1671	1673	0.001 ± 0.001	0.2 ± 0.1	
73	n-tetradecanol	OI	1671	1679	0.022 ± 0.016	1 ± 1	
74	cyclotetradecane	HC	1673	1681	0.006 ± 0.004	0.4 ± 0.4	
75	heptadecane	HC	1700	1705	0.005 ± 0.005	1.0 ± 0.8	
76	unk 6	HC	-	1710	0.05 ± 0.03	0.4 ± 0.3	
77	unk 7	Arom	-	1715	0.005 ± 0.005	0.4 ± 0.4	
78	methyl tetradecanoate	Ester	1725	1730	0.10 ± 0.06	0.4 ± 0.4	
79	4-methyl-heptadecane	HC	1758	1759	0.07 ± 0.03	0.2 ± 0.1	
80	2-methyl-heptadecane	HC	1765	1764	0.08 ± 0.04	0.05 ± 0.04	
81	3-methyl-heptadecane	HC	1770	1771	0.08 ± 0.03	0.12 ± 0.08	
82	octadecane	HC	1800	1795	0 ± 0	0.8 ± 0.6	
83	unk HC 2	HC	-	1804	0.12 ± 0.06	0.6 ± 0.5	

n	Compound	Class[a]	ARi_{rep}[b]	ARi_{calc}[c]	Undamaged plants[d]	*T. absoluta*-damaged plants[d]	t test (p value)
84	hexadecanal	Ald	1817	1812	0.03 ± 0.02	0.7 ± 0.6	
85	6,10,14-trimethyl-2-pentadecanone	One	1844	1840	0.243 ± 0.05	0.7 ± 0.4	
86	2-methyl-octadecane	HC	1863	1858	0 ± 0	0.2 ± 0.2	
87	unk 8	NI	-	1871	0.04 ± 0.02	0.2 ± 0.2	
88	1-hexadecanol	Ol	1880	1875	0.1 ± 0.1	3 ± 3	
89	unk 9	NI	-	1882	0.06 ± 0.03	0.2 ± 0.2	
90	nonadecane	HC	1900	1893	0 ± 0	0.6 ± 0.5	
91	unk 10	NI	-	1896	0.04 ± 0.02	0.2 ± 0.1	
92	unk 11	NI	-	1913	0.04 ± 0.01	0.20 ± 0.08	
93	methyl hexadecanoate	Ester	1921	1921	0.01 ± 0.01	0.6 ± 0.4	
94	unk 12	NI	-	1982	0.08 ± 0.07	0.3 ± 0.2	
95	1-eicosene	HC-ene	1987	1996	0.01 ± 0.01	0.3 ± 0.3	
96	eicosane	HC	2000	1999	0.06 ± 0.04	0.3 ± 0.2	
97	octadecanal	Ald	2021	2023	0.01 ± 0.01	0.3 ± 0.2	
98	isopropyl palmitate	Ester	2023	2027	0.05 ± 0.03	0.10 ± 0.07	
99	*E,E*-geranyl linalool	DT	2026	2033	0.13 ± 0.05	0.6 ± 0.3	0.004
100	(*Z*)-9-octadecen-1-ol	Ol	2063	2063	0.4 ± 0.2	2 ± 1	
101	n-octadecanol	NI	2077	2088	0.05 ± 0.05	2 ± 1	
102	heneicosane	HC	2100	2103	0 ± 0	0.3 ± 0.3	
103	docosane	HC	2200	2199	0 ± 0	0.1 ± 0.1	

[a]: Ald: Aldehyde; Arom: Aromatic; DT: Diterpene; Ester: Ester; HC: hydrocarbon; HC-ene: hydrocarbon-alkene; MeSa: methyl salicylate; MT: monoterpene; NI: Unidentified; Ol: Alcohol; ST: Sesquiterpene; St m: modified sesquiterpene. [b]: ARi_{rep}: Retention index reported in NIST 05 (Linstrom and Mallard 2005) and Adams (Adams 2007) mass spectrum libraries. [c]: Retention index calculated. [d]: µg eq IS ± SE. *unk: Unknown.

Table 2
Volatiles emitted by undamaged and *T. vaporariorum*-damaged tomato plants (N = 13 pairs of undamaged/damaged plants).

n	Compound	Class[a]	ARi_{rep}[b]	ARi_{calc}[c]	Undamaged plants[d]	*T. vaporariorum*-damaged plants[d]	t test (p value)	VIP > 1 PLSDA
1	n-octane	HC	800	803	0.04 ± 0.02	0.004 ± 0.001		
2	n-nonane	HC	900	900	0.002 ± 0.001	0.02 ± 0.01		
3	α-pinene	MT	932	931	0.11 ± 0.05	0.1 ± 0.03		
4	tert-butyl-benzene	Arom	976	969	0.10 ± 0.02	0.06 ± 0.03		
5	β-pinene	MT	979	974	0.05 ± 0.03	0.05 ± 0.02		
6	2,2,4,6,6-pentamethyl-heptane	HC	991	989	2.6 ± 0.4	1.8 ± 0.3		
7	unk* HC 3	HC	-	991	0.53 ± 0.09	0.24 ± 0.04		2.067
8	δ2-carene	MT	1001	1000	1.3 ± 0.2	0.5 ± 0.2	< 0.001	1.190
9	α -phellandrene	MT	1002	1003	0.20 ± 0.03	0.14 ± 0.04		
10	α -terpinene	MT	1014	1016	0.010 ± 0.003	0.005 ± 0.002		
11	p-cymene	MT	1020	1024	0.16 ± 0.05	0.05 ± 0.01		1.145
12	β-phellandrene	MT	1025	1028	6 ± 1	2.6 ± 0.6	< 0.001	2.879
13	3,7-dimethyl-nonane	HC	1038	1034	0.40 ± 0.09	0.23 ± 0.05		
14	(*E*)β-ocimene	MT	1044	1048	0.32 ± 0.06	0.17 ± 0.03		1.075
15	γ-terpinene	MT	1054	1058	0.06 ± 0.02	0.03 ± 0.006		
16	terpinolene	MT	1086	1089	0.030 ± 0.004	0.015 ± 0.002		
17	n-undecane	HC	1100	1100	0.04 ± 0.01	0.057 ± 0.008		
18	n-nonanal	Ald	1100	1105	0.10 ± 0.01	0.08 ± 0.01		
19	unk 13	NI	-	1116	0.10 ± 0.01	0.12 ± 0.01		
20	methyl salicylate	MESa	1190	1195	0.13 ± 0.08	0.05 ± 0.03		
21	dodecane	HC	1200	1200	0.028 ± 0.009	0.014 ± 0.004		
22	n-decanal	Ald	1201	1207	0.08 ± 0.02	0.049 ± 0.008		
23	2,6,11-trimethyl-dodecane	STm	1275	1282	0.071 ± 0.008	0.044 ± 0.007		
24	1-tridecene	Hc-ene	1290	1290	0.09 ± 0.03	0.08 ± 0.02		
25	undecanal	Ald	1305	1310	0.10 ± 0.02	0.029 ± 0.005		1.791
26	δ-elemene	ST	1335	1339	0.05 ± 0.01	0.03 ± 0.01		1.410

n	Compound	Class[a]	ARi_{rep}[b]	ARi_{calc}[c]	Undamaged plants[d]	*T. vaporariorum*-damaged plants[d]	t test (p value)	VIP > 1 PLSDA
27	2-methyl-tridecane	HC	1364	1364	0.16 ± 0.05	0.07 ± 0.02		
28	3-methyl-tridecane	HC	1371	1371	0.19 ± 0.05	0.09 ± 0.03		
29	2-methyl-3-hydroxy-2,2,4-trimethylpentyl-ester-propanoic acid	Ester	1380	1375	0.04 ± 0.02	0.015 ± 0.003		
30	farnesane	ST	1366	1377	0.17 ± 0.05	0.07 ± 0.02		1.003
31	tetradecane	HC	1400	1400	0.55 ± 0.09	0.06 ± 0.04		2.366
32	*e*-caryophyllene	ST	1417	1421	0.3 ± 0.1	0.13 ± 0.04		1.450
33	octyl cyclohexane	HC	1448	1447	0.20 ± 0.05	0.06 ± 0.02		1.626
34	unk 14	ST	-	1449	0.22 ± 0.06	0.07 ± 0.03		1.516
35	2,6,10-trimethyltridecane	STm	1449	1451	0.24 ± 0.06	0.09 ± 0.03		1.436
36	5-methyl-tetradecane	HC	1453	1454	0.31 ± 0.07	0.12 ± 0.03		1.123
37	a-humulene	ST	1452	1456	0.15 ± 0.04	0.07 ± 0.01		
38	4-methyl-tetradecane	HC	1459	1460	0.25 ± 0.07	0.08 ± 0.03		
39	2-methyl-tetradecane	HC	1463	1463	0.56 ± 0.14	0.17 ± 0.07		2.307
40	3-methyl-tetradecane	HC	1470	1472	0.3 ± 0.1	0.13 ± 0.04		1.260
41	unk 15	ST	-	1476	0.11 ± 0.04	0.042 ± 0.006		
42	unk 16	HC-ene	-	1482	0.8 ± 0.02	0.046 ± 0.006		
43	unk 17	NI	-	1484	0.07 ± 0.02	0.043 ± 0.007		
44	1-pentadecene	Hc-ene	1492	1496	0.13 ± 0.02	0.04 ± 0.01		
45	2-hexyl-1-decanol	Ol	1504	1497	0.4 ± 0.1	0.21 ± 0.06		1.888
46	pentadecane	HC	1500	1502	0.8 ± 0.2	0.1 ± 0.1		2.762
47	tridecanal	Ald	-	1507	0.15 ± 0.04	0.06 ± 0.01		
48	unknown alcohol 1	Ol	1504	1511	0.35 ± 0.08	0.16 ± 0.03		
49	unk 18	NI	-	1517	0.19 ± 0.03	0.09 ± 0.02		
50	unk 19	NI	-	1542	0.29 ± 0.04	0.15 ± 0.02		1.619
51	(3*E*,7*E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene	STm	1577	1581	0.4 ± 0.2	0.5 ± 0.1		
52	hexadecane	HC	1600	1599	0.9 ± 0.2	0.43 ± 0.08		1.100
53	tetradecanal	Ald	1611	1614	0.12 ± 0.03	0.07 ± 0.01		
54	heptadecane	HC	1700	1690	0.5 ± 0.1	0.35 ± 0.04		
55	2,6,10-trimethyl-hexadecane	STm	1727	1715	0.32 ± 0.07	0.17 ± 0.02		

n	Compound	Class[a]	ARi_{rep}[b]	ARi_{calc}[c]	Undamaged plants[d]	*T. vaporariorum*-damaged plants[d]	t test (p value)	VIP > 1 PLSDA
56	unk 20	NI	-	1719	0.17 ± 0.03	0.11 ± 0.02		
57	unk 21	NI	-	1724	0.5 ± 0.1	0.42 ± 0.04		
58	unk 22	NI	-	1750	0.26 ± 0.06	0.13 ± 0.01		
59	unk 23	NI	-	1754	0.17 ± 0.08	0.022 ± 0.006		1.059
60	unk 24	NI	-	1760	0.12 ± 0.07	0.06 ± 0.02		
61	4-methyl-heptadecane	HC	1758	1764	0.11 ± 0.03	0.1 ± 0.01		
62	2-methyl-heptadecane	HC	1765	1768	0.12 ± 0.03	0.078 ± 0.008		
63	3-methyl-heptadecane	HC	1770	1775	0.10 ± 0.03	0.058 ± 0.007		
64	octadecane	HC	1800	1799	0.21 ± 0.06	0.17 ± 0.01		
65	(*Z*)-7-hexadecenal	Ald	1798	1808	0.17 ± 0.06	0.08 ± 0.01		
66	unk 25	NI	-	1826	0.03 ± 0.01	0.016 ± 0.007		
67	isopropyl myristate	Ester	1827	1828	0.14 ± 0.02	0.07 ± 0.02		1.308
68	unk 26	NI	-	1848	0.08 ± 0.02	0.039 ± 0.004		
69	n-hexadecanol	OI	1800	1883	0.34 ± 0.08	0.3 ± 0.1		
70	unk alcohol 2	OI	-	1886	0.09 ± 0.04	0.07 ± 0.01		
71	unk 27	NI	-	1891	0.07 ± 0.03	0.056 ± 0.008		
72	unk 28	NI	-	1895	0.08 ± 0.06	0.008 ± 0.002		
73	nonadecane	HC	1900	1899	0.3 ± 0.2	0.094 ± 0.008		
74	unk 29	NI	-	1903	0.02 ± 0.01	0.009 ± 0.001		
75	unk 30	NI	-	1907	0.014 ± 0.006	0.022 ± 0.003		
76	unk 31	NI	-	1917	0.3 ± 0.2	0.016 ± 0.006		
77	unk 32	NI	-	1920	0.2 ± 0.1	0.004 ± 0.003		1.139
78	unk 33	NI	-	1924	0.4 ± 0.3	0.011 ± 0.004		1.600
79	methyl hexadecanoate	HC	1921	1928	0.04 ± 0.02	0.06 ± 0.01		1.172
80	unk 34	NI	-	1932	0.05 ± 0.05	0.003 ± 0.002		
81	unk 35	NI	-	1935	0.06 ± 0.04	0.002 ± 0.001		
82	unk 36	NI	-	1947	0.5 ± 0.5	0.009 ± 0.004		1.082
83	unk 37	NI	-	1960	0,008 ± 0,006	0.002 ± 0.001		
84	eicosane	HC	2000	1999	0.2 ± 0.1	0.057 ± 0.006		
85	unk 38	NI	-	2023	0,019 ± 0,008	0.006 ± 0.002		

n	Compound	Class[a]	ARi_{rep}[b]	ARi_{calc}[c]	Undamaged plants[d]	*T. vaporariorum*-damaged plants[d]	t test (p value)	VIP > 1 PLSDA
86	isopropyl palmitate	Ester	2023	2028	0,11 ± 0,03	0.04 ± 0.01		
87	unk 39	NI	-	2035	0.011 ± 0.007	0.018 ± 0.005		
88	(*Z*)-9-octadecen-1-ol	OI	2063	2065	0.09 ± 0.03	0.11 ± 0.07		
89	n-octadecanol	OI	2077	2089	0.13 ± 0.03	0.13 ± 0.03		
90	heneicosane	HC	2100	2102	0.015 ± 0.004	0.026 ± 0.004		
91	docosane	HC	2200	2199	0.011 ± 0.003	0.012 ± 0.002		
92	unk 40	NI	-	2410	0.03 ± 0.03	0.001 ± 0.0001		

[a]: Ald: Aldehyde; Arom: Aromatic; Ester: Ester; HC: hydrocarbon; HC-ene: hydrocarbon-alquene; MeSa: methyl salicylate; MT: monoterpene; NI: Unidentified; OI: Alcohol; ST: Sesquiterpene; St m: modified sesquiterpene. [b]: ARi_{rep}: Retention index reported in NIST 05 (Linstrom and Mallard 2005) and Adams (Adams 2007) mass spectrum libraries. [c]: Retention index calculated. [d]: µg eq IS ± SE. *unk: Unknown.

Table 3
Volatile analysis overview of undamaged and damaged tomato plants.

Experiment	***T. absoluta* damage**			***T. vaporariorum* damaged**		
Variable	**Undamaged plants**	*T. absoluta*-**damaged plants**	Wilcoxon test (p value)	**Undamaged plants**	*T. vaporariorum*-**damaged plants**	Wilcoxon test (p value)
Maximum number of peaks	104	104	-	92	92	-
Number of peaks identified	92	92	-	64	64	-
Mass of volatiles emitted (mean ± SE µg eq of IS**)**	22 ± 5	56 ± 5	0.05	26 ± 7	13 ± 3	0.002
Compound groups:			Paired t-test (p value)			Paired t-test (p value)
Alcohols	2 ± 2	8 ± 6	ns	1.4 ± 0.2	1.0 ± 0.3	ns[a]
Aldehydes	0.10 ± 0.09	3 ± 3	ns	0.7 ± 0.2	0.4 ± 0.1	ns
Aromatic Compounds	0.10 ± 0.02	0.7 ± 0.5	0.005	0.10 ± 0.02	0.07 ± 0.03	0.01
Diterpenes	0.13 ± 0.04	0.6 ± 0.3	0.003	-	-	-
Esters	0.3 ± 0.2	4 ± 3	0.02	0.28 ± 0.04	0.14 ± 0.03	ns
Hydrocarbons saturated	1.1 ± 0.6	8 ± 6	ns	10 ± 1	5.1 ± 0.8	0.02
Hydrocarbons unsaturated	0.02 ± 0.02	0.4 ± 0.3	ns	0.25 ± 0.07	0.18 ± 0.04	ns
Indole	0.011 ± 0.007	0.06 ± 0.03	0.001	-	-	-
Ionone	0.03 ± 0.01	0.3 ± 0.1	< 0.001	-	-	-
Ketones	0.24 ± 0.05	0.7 ± 0.4	ns	-	-	-
Methyl Salicylate	0.05 ± 0.03	0.18 ± 0.08	0.02	0.13 ± 0.08	0.05 ± 0.04	ns
Modified Sesquiterpenes	3 ± 1	6 ± 2	ns	1.0 ± 0.2	0.7 ± 0.2	ns
Monoterpene hydrocarbons	11 ± 3	12 ± 3	ns	8 ± 1	3.8 ± 0.9	< 0.001
Monoterpenes oxygenated	0.003 ± 0.002	0.011 ± 0.006	ns	-	-	-
Sesquiterpene hydrocarbons	3 ± 1	9 ± 4	ns	1.0 ± 0.2	0.37 ± 0.1	< 0.001

[a]: ns, non significant

Experiment	*T. absoluta* damage			*T. vaporariorum* damaged		
Unidentified	0.5 ± 0.3	4 ± 3	ns	4 ± 1	1.4 ± 0.2	ns

[a]: ns, non significant

In the case of *T. absoluta*-damaged plants, despite the similarity in the qualitative analysis, there was a tendency to increase volatile emissions (56 ± 5 in damaged plants *vs.* 22 ± 5 in undamaged plants -µg eq IS ± SE; p = 0.05, Wilcoxon test, Table 3). Of the 103 quantified peaks, 91 of them were successfully identified (Tables 1 and 3). Ten out of the 103 compounds were emitted only by damaged plants. On the other hand, only one unidentified compound, Unk3, was emitted in undamaged plants but not in damaged ones (Table 1). Univariate analyses on the 103 compounds showed that 12 of them varied when comparing the volatiles from undamaged and damaged plants (Table 1, paired *t-tests*, $p < 0.05$). These 12 compounds included 1 monoterpene (carvacrol), 1 aldehyde (2*E*-decenal), 2 sesquiterpenes (*E*-caryophyllene, α-humulene), 1 diterpene (*E,E*-geranyl linalool), 1 hydrocarbon (tetradecane), 1 alcohol (2-hexyl-1-decanol), 2 esters (3*Z*-hexenyl butanoate), 2 aromatics (indole, benzophenone), and the carotenoid (*E*)-β-ionone.

Multivariate analyses were performed on the matrix generated (103 compounds x 2 plant treatments), with previous data normalization (Log_{10}) and scaling (Pareto scaling) (Alaerts 2010). First, the PCA (singular value decomposition, Fig. S2) showed that the data was well explained (80% of variance) by 5 components (Component 1: 49%; Component 2: 16%; Component 3: 6%; Component 4: 5%; Component 5: 4%). When modeling these data by the PLS-DA, the model has a p value > 0.1 in the permutation tests, so no conclusion could be drawn from this analysis (Fig. S3). Worth noticing, a Random Forest analysis correctly classified samples from the two plant treatments with an OOB error = 0.11 (precision 92% and 85% for damaged and undamaged plants respectively). All these data together show that volatiles emitted by undamaged plants present some compounds in significantly different amounts than volatiles from plants damaged by *T. absoluta*.

In the case of *T. vaporariorum*-damaged plants, there was a significant reduction in the total amount of volatiles emitted after damage (26 ± 7 *vs.* 13 ± 3 µg eq IS ± SE, p = 0.002, Wilcoxon *test*). Of the 92 quantified peaks, 64 of them were successfully identified (Tables 2 and 3). In this case, all 92 peaks were detected in both kinds of plants, in different amounts. Univariate analyses on the 92 compounds showed that 2 of them varied when comparing the volatiles from undamaged and damaged plants: δ2-carene and β-phellandrene (Table 2, paired *t-tests*, $p < 0.05$). Multivariate analyses were then performed on the matrix generated (92 Compounds x 2 kind of plants). Previous to statistical analysis, these data were normalized (square root), and scaled (Pareto scaling) (Alaerts 2010). First, the PCA (singular value decomposition, Fig. S4) showed that the data was well explained (67% of variance) by 5 components (Component 1: 28%; Component 2: 14%; Component 3: 12%; Component 4: 7%; Component 5: 6%). Then a partial least squares-discriminant analysis (PLS-DA, Fig. 3) was used to model the differences between undamaged and damaged plants. Permutation tests based on separation distance were applied to evaluate the reliability of the model (2000 permutations, p = 0.04). Overall, the PLS-DA model was found to be an acceptable model for discrimination between the plant status. The validated model had 5 components, with $R^2 = 0.95$, $Q^2 = 0.85$ and accuracy of 1 (Fig. 3). From the model built, 26 compounds with a Variable Importance in Projection (VIP) greater than 1 (Chong et al. 2018) were found (Table 2). Nineteen out of these 26 were identified (Table 2): 4 were monoterpenes (δ2-carene, p-cymene, β-phellandrene, (*E*)-β-ocimene), 1 aldehyde (Undecanal), 4 sesquiterpenes (δ-elemene, farnesane, E-caryophyllene, 2,6,10-trimethyltridecane -modified ST-), 7 hydrocarbons (tetradecane, octyl-cyclohexane, 5-methyl-tetradecane, 2-methyl-tetradecane, 3-methyl-tetradecane, pentadecane, hexadecane), 1 alcohol (2-hexyl-1-decanol) and 2 esters (isopropyl myristate, methyl hexadecanoate). Finally, Random Forest analyses correctly classified samples from the two plant statuses with an OOB error = 0.08 (precision 85% and 100% for damaged and undamaged plants respectively). All these data together also clearly show that the volatiles from undamaged plants can be differentiated from the volatiles from species damaged by *T. vaporariorum*.

Insert Fig. 3*here*

DISCUSSION

Tuta absoluta females laid more eggs on conspecific-damaged plants in two-choice bioassays with undamaged plants as an option. These results may be due to different plant chemistry, either volatile or non-volatile, resulting from the previous damage by conspecifics. Our volatile analysis in tomato plants (discussed later) did show significant changes due to *T. absoluta* damage, but we cannot rule out other possible effects, chemical or otherwise, related to the presence of *T. absoluta* larvae feeding on the plants. Beyond the mechanistic explanation, the oviposition preference we found may be also discussed in adaptive terms. It has been shown that lepidopteran larvae may benefit from developing together, concerning both biotic and abiotic stressors (Tsubaki 1981). Moreover, aggregation of lepidopteran larvae (Jin et al. 2016; Jumean 2004; Tsubaki 1981) and attraction of females to oviposit (Sun et al. 2014) on plant areas with presence of conspecifics have been previously reported for different families. While *T. absoluta* females lay eggs singly, several larvae usually coexist in the same plant, so potential adaptive explanations for *T. absoluta* females to prefer ovipositing on conspecific-damaged plants are not unforeseen.

Our oviposition preference results differ from previous studies that reported *T. absoluta* females ovipositing more eggs on undamaged plants in comparison with conspecific-damaged ones (Anastasaki et al. 2018; Bawin et al. 2014; Maneesha et al. 2021). These contradictory results may stem from methodological differences or even from plant cultivar characteristics. Bawin et al. (2014) found oviposition preferences for undamaged plants but no volatile-mediated preferences in wind tunnel assays, pointing to other plants cues such as non-volatile induced defenses. Maneesha et al. (2021) did not report experimental conditions such as the size of the bioassay cages, larval infestation levels or whether damaging larvae were retrieved before the preferences assays, all factors that may have influenced the preference results. Anastasaki et al. (2018) worked with preference bioassays using shorter distances (60 cm *vs.* 120 cm) and smaller containers than ours (2.1 L vs. 3.6 L), which may have influenced the results due to the closeness of the contrasting plants. Infestation levels was also different both in the number and instar of damaging larvae: twenty larvae (L1) were used by Anastasaki et al. (2018), while we used 25 older larvae (L3), likely increasing the level of damage and induced response by the plant. Indeed, It has been reported that the age of *T. absoluta* larvae feeding on tomato plants affects adult attraction to the plants (Abdelhady 2020). All in all, different results may arise from different experimental conditions or cultivars; San Pedro in our study, Moneymaker in Bawin et al. (2014), Semiramis in Anastasaki et al. (2018) and Sahoo TO-3251 in Maneesha's work (2021). Different cultivars are not only reported to impact in *T. absoluta* fitness (Mathieu W. Sawadogo et al. 2021) and oviposition preferences (Cherif 2013; Proffit et al. 2011), but also differ in the volatiles emitted (see below) as well as in the defensive secondary metabolites and trichome density, which are known to play a role in tomato resistance to *T. absoluta* (Sohrabi et al. 2016) and differentially affect *T. absoluta* oviposition behavior (Oliveira et al. 2012; Regina Gontijo Labory C 1999).

Our results also showed that *Trialeurodes vaporariorum* preferred to settle on plants with previous damage by conspecifics. This is in line with results with *B. tabaci* females, which prefer to lay on plants previously occupied by conspecifics (Silva et al. 2021a; Su et al. 2018). Previous olfactometer studies with *T. vaporariorum* adults, however, showed conflicting results. *Trialeurodes vaporariorum* adults were more attracted to volatiles emitted by undamaged plants than to those from conspecific-damaged plants. This was the case for two tomato cultivars, but did not hold for two other cultivars (Deletre et al. 2022) (none of these cultivars are the same as in this study). In a different study, *T. vaporariorum* males were more attracted to conspecific-infested tomato plants than to undamaged ones, but females showed the opposite results (Darshanee et al. 2017). Therefore, attraction to undamaged over damaged plants for *T. vaporariorum* depends on plant cultivar and whitefly sex. In our work, experimental *T. vaporariorum* adults were not separated for sex, so we cannot rule out that the settling preference we found was not affected by sex ratio of the tested insects.

Our findings of *T. vaporariorum* settling preferences may also have adaptive implications. It has been shown that whiteflies may benefit from conspecific feeding aggregations (facilitation) via sink modification, a mechanism by which whiteflies can control the sap flow within the plant to their advantage. This in turn may reduce the nutritional quality of the plants to other competing herbivores, especially non-sap-feeders (Inbar and Gerling 2008). In addition, whitefly feeding has been reported to

suppress the effects of the JA pathway by activation of the SA pathway (Zhang et al. 2013), which may also benefit the formation of aggregations.

In our crossed herbivore preference bioassays, *T. absoluta* preferred ovipositing on *T. vaporariorum* damaged plants, and *T. vaporariorum* preferred settling on *T. absoluta* damaged plants. Partly in line with our results, *T. absoluta* larval development was positively affected by the previous presence of *B. tabaci*, but only on locally-damaged leaves and not when the damage occurred in different leaves (Mouttet et al. 2013). This effect was not symmetrical, since previous infestation by *T. absoluta* did not affect the development of *B. tabaci* nymphs (Mouttet et al., (2013). Our results are also in line with a study with the leaf-chewer *Pieris brassicae* (Lepidotera: Pieridae) and the phloem-sucking aphid *Brevicoryne brassicae* (Hemiptera, Aphididae), which showed better performance of the larvae on heterospecific damaged cabbage plants, in comparison with undamaged plants (Soler et al. 2012).

The difficulty for the plant to defend itself when it has been already attacked by a pest that activates a different defensive route has already been studied in various systems in relation to the crosstalk effect between hormone-controlled pathways (2012). The previous results by Mouttet (2013) rise the hypothesis that the tomato plants are able to defend itself at the same time locally to herbivore damages but not systemically for both herbivores. A similar scenario may allow *T. absoluta* and *T. vaporariorum* coexistence. No oviposition preference of *T. absoluta* has been previously documented, as far as we know, related to the previous presence of *T. vaporariorum*.

To sum up, our laboratory findings did indeed confirm the co-occurrence that was previously observed in local greenhouses. In the case of *T. vaporariorum*-damaged plants, preference would be caused by a downregulation of plant indirect defenses as happens when *B. tabaci* damages the tomato plant (Zhang et al. 2019). In the case of *T. absoluta*-damaged plants, more studies are needed to elucidate the underlining mechanisms that favor the whitefly settling. The differences found compared to previous reports, and among them, may be explained due to the cultivars of tomato plants used, the distance between the stimuli, the number of larvae of the tomato leafminer or nymphs or adult whiteflies causing damage, among other effects. Besides, since damaging insects were not removed, we cannot rule out that attraction to damaged plants may be also influenced by some insect cue. Although, in the case of *T. vaporariorum* attraction to conspecifics seems not to take place (Darshanee et al. 2017).

Regarding volatiles emitted by the tomato plants here studied, as mentioned, *T. absoluta* damaged plants emitted more volatiles after the damage (Table 3), as it was previously reported (Anastasaki et al. 2018; Ayelo et al. 2021a; Chen et al. 2021; Silva et al. 2018; Silva et al. 2017). Among the many reports on *T. absoluta*-damaged plants volatiles, although the main identified compounds are similar among works, there is an enormous variation on the reported compounds (Anastasaki et al. 2015; Anastasaki et al. 2018; Ayelo et al. 2021a; Caparros Megido et al. 2014; Chen et al. 2021; De Backer et al. 2015; Gontijo et al. 2019; Milonas et al. 2019; Proffit et al. 2011; Silva et al. 2018; Silva et al. 2017). In these works, together, more than 200 compounds are reported, of which none of them are common to all works, and for instance, only 32 (16%) are reported in at least four of them (considering the works where all compounds are listed). The different tomato cultivars, soil, location and season of the year in which the volatiles collections are done are some of the factors that may explain these differences in volatile profiles (Holopainen and Gershenzon 2010). Except for the work by Chen et al. (2021), none of the other publications reviewed here quantify more than 60 compounds. In this work, 103 peaks (91 identified) are reported, allowing to show change in minor peaks after the damage. Among the 12 compounds found here to vary significantly in *T. absoluta*-damaged plants, *E*-caryophyllene was previously reported to cause a physiological response in *T. absoluta*'s antennae (Anastasaki et al. 2018; Miano RN 2022; Pagadala Damodaram et al. 2021), and to increase significantly its amount after the damage (Anastasaki et al. 2018; Ayelo et al. 2021a; Ayelo et al. 2021b; Maneesha et al. 2021; Silva et al. 2018), as in our results (Table 2). However, in another report, even if *E*-caryophyllene was detected in HIPVs from *T. absoluta*-damaged plants, its amount did not vary after being damaged just as the amount of the rest of the sesquiterpenes did not vary either (De Backer et al. 2015). Tetradecane (Maneesha et al. 2021), (*Z*)-3-hexenyl butanoate (Ayelo et al. 2021a), Indole (Silva et al. 2017), (*E*)-β-ionone and α-humulene (Ayelo et al. 2021a; Silva et al. 2018) were also previously reported to increase significantly when the tomato plants are damaged by *T. absoluta* larvae. Besides, α-Humulene and Undecane were

also previously reported to cause a physiological response in *T. absoluta*'s antennae (Pagadala Damodaram et al. 2021) although Undecane was not reported to increase after damage, as it did in our work. However, in the case of Tetradecane and (*Z*)-3-hexenyl butanoate, no antennal response to them was detected (Anastasaki et al. 2018). The other five compound (2-*E*-Decenal, Carvacrol, 2-hexyl-1-Decanol, Benzophenone and *E*, *E*-Geranyl linalool, Table 1) that vary significantly their emision after *T. absoluta* damage in tomato plants were not previously reported in any of the above-mentioned works.

Damaged plants by *T. vaporariorum* emitted less volatiles than undamaged tomato plants (Table 2), similarly to the previously reported not only for *T. vaporariorum* (Deletre et al. 2022) but also for *B. tabaci* (Silva et al. 2017). However, this pattern is not general as it has been also reported that HIPVs after *T. vaporariorum* damage increased when tomato plants were infested by 100 whitefly adults (Ayelo et al. 2021c). Interesting, this last work also showed that the emission of HIPVs decreased when infestation was higher (with 200 adults) (Ayelo et al. 2021c). In the case of *B. tabaci*-damaged tomato plants, an increase in HIPVs has also been reported (Silva et al. 2021a). As mentioned, tomato volatile characterization has been previously reported not only for *T. vaporariorum* damage (Ángeles López et al. 2012; Ayelo et al. 2021c; Darshanee et al. 2017; Deletre et al. 2022) but also for *B. tabaci*'s (Chen et al. 2020; Silva et al. 2018; Silva et al. 2021a; Silva et al. 2017; Su et al. 2018). Among these reports and ours, more than 150 compounds were quantified. Of the 26 compounds that were here found to vary significantly after damage by *T. vaporariorum*, only three compounds, (*E*)-β-ocimene, terpinolene and *E*-caryophyllene are quantified in all reports. δ2-Carene and β-phellandrene, were previously reported to decrease significantly after damage by *T. vaporariorum* (Ángeles López et al. 2012). β-Phellandrene also decreased in four tomato cultivars after *T. vaporariorum*'s damage (Deletre et al. 2022; Silva et al. 2018) but increased after *B. tabaci*'s ((Chen et al. 2020; Silva et al. 2018). On the other hand, *E*-Caryophyllene and (*E*)-β-Ocimene increased significantly in the different works (Ángeles López et al. 2012; Ayelo et al. 2021c; Silva et al. 2018). Although *E*-Caryophyllene increased after damage of 100 whiteflies, decreased after damage of 200 whiteflies. p-Cymene increased in the only work that was quantified and was also reported to be repellent for *T. vaporariorum* (Ayelo et al. 2021c). Our results showed that p-Cymene and (*E*)-β-ocimene decreased significantly, and adults were attracted to damaged plants (see above). One wonders whether the decrease in repellent compounds (Deletre et al. 2022) may favor the settling of the whiteflies. δ-Elemene was quantified in two publications and in both it did not vary significantly (Ayelo et al. 2021b; Silva et al. 2018). The four compounds just mentioned that vary plus δ2-Carene and hexadecane are reported to cause a physiological response in *T. absoluta*'s antennae (Anastasaki et al. 2018; Chen et al. 2021; Miano RN 2022; Pagadala Damodaram et al. 2021). The other 18 compounds that vary significantly in our work were not quantified in any of the above-reviewed reports. This comparative analysis shows the huge variation among works in volatiles emitted by whiteflies-damaged tomato plants. This variation is such that in some works volatiles are up-regulated and in others are down-regulated, either as whole or individually. Given that, at least in the case of *T. vaporariorum*, the gregariousness does not respond to own cues (Darshanee et al. 2017), it is highly probable that the stimuli of the plants contribute to the gathering of the insects. Clearly then, further studies like olfactometry assays and electroantennography analyses are needed to understand the role of these compounds.

Comparing the variation of volatiles emitted after been damaged by *T. absoluta* and *T. vaporariorum*, as mentioned, 12 and 26 compounds vary significantly respectively, but only two of them, *E*-Caryophyllene and tetradecane, vary in both cases. Both compounds increased after *T. absoluta* damage and decreased after *T. vaporariorum*'s. Having both insects different feeding habits (Pieterse et al. 2012), they would activate different defensive routes in tomato plants (Walling 2000), leading probably to different variations in volatiles. Since both insects were attracted to plants damaged by conspecific and heterospecific individuals, which exhibited different volatile variations, the attractiveness to damaged plants could be guided by the mixture of compounds rather than by any of them individually, and by the balance with other non-volatile stimuli present.

CONCLUSIONS

In this study, *T. absoluta* and *T. vaporariorum* preferred laying eggs or settling respectively on damaged plants by either a conspecific or the other herbivore. Our results on the insects´ preferences agree with both, our own field observation and with

previous reports in the case of *T. vaporariorum*. However, the oviposition preference observed for *T. absoluta* differs from some previous studies. This difference probably arises from different experimental conditions and tomato cultivars used in the studies. The preference for conspecific-damaged plants may be driven by gregariousness or a general decrease in plant defenses following an attack. On the other hand, the preference for heterospecific-damaged plants might be due to the challenge plants face in defending themselves against simultaneous attacks from both insects, as reported in other systems. The compounds emitted by the tomato plants varied in a different way when attacked by one or the other insect; and differed also from the variations of individual compounds previously reported in tomato HIPVs. Interestingly, some individual compounds showed opposing variations under the damage caused by both herbivores. Nonetheless, plants emitting these compounds remained consistently attractive to the herbivores. This suggests a hypothesis that preferences are determined by the mixture of volatiles rather than individual compounds, as well as possibly modulated by other stimuli. To establish a definitive conclusion on whether this chemical variation in volatiles governs insects' choice, further studies utilizing olfactometer preference bioassays and wind tunnel experiments are warranted, as understanding the behavior of these herbivores (and the underlying cues) in the presence of other one is essential for designing a strategy to control them.

Declarations

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Figures

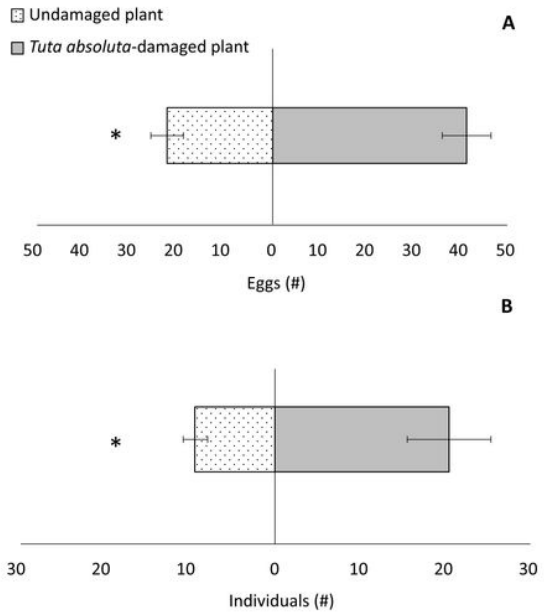

Figure 1

Oviposition preference of *T. absoluta* (**A**, N = 12) and settling preference of *T. vaporariorum adults* (**B**, N = 11) after 72h between undamaged and *T. absoluta*-damaged plants in choice experiments (numbers of eggs and individuals respectively). * denotes significant differences ($p < 0.05$, Wilcoxon tests; results shown as mean ± standard error).

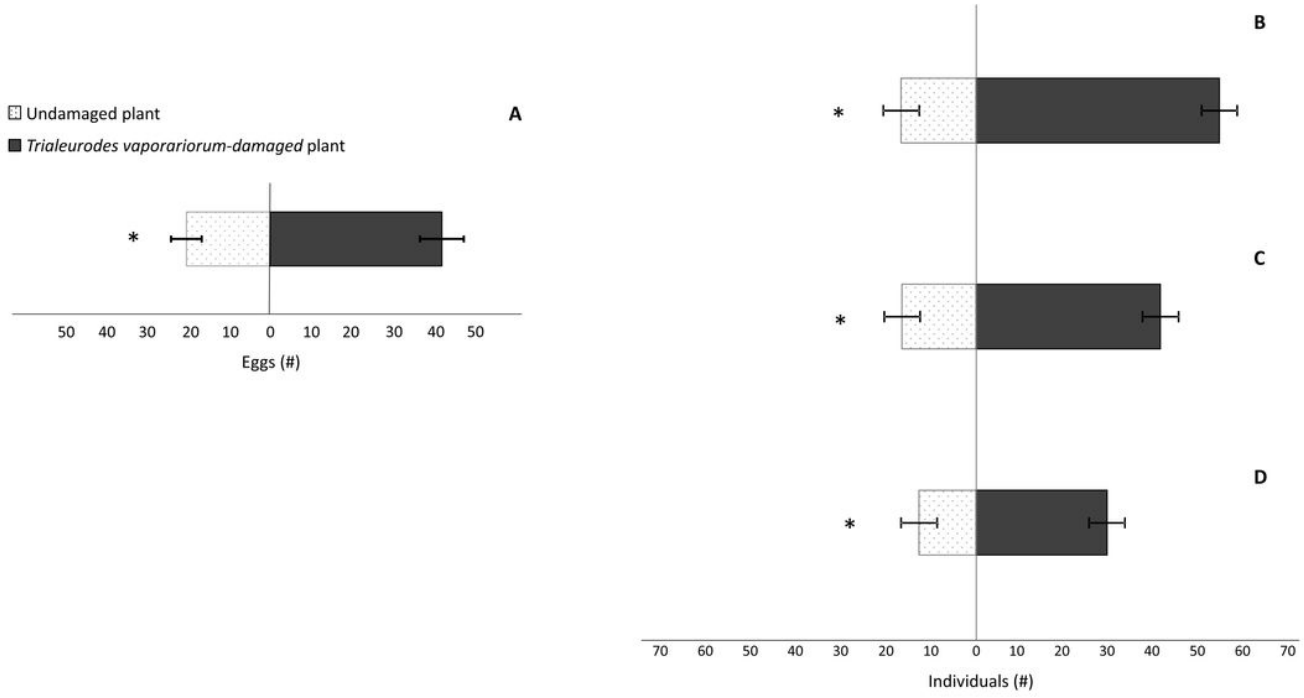

Figure 2

Oviposition preference of *T. absoluta* (**A**,N = 7) and settling preference of *T. vaporariorum adults* (N = 10) after 24h (**B**), 48h (**C**) and 72h (**D**) between undamaged and *T. vaporariorum*-damaged plants in choice experiments (* denotes significant differences at $p < 0.05$,Wilcoxon tests; results shown as mean ± standard error).

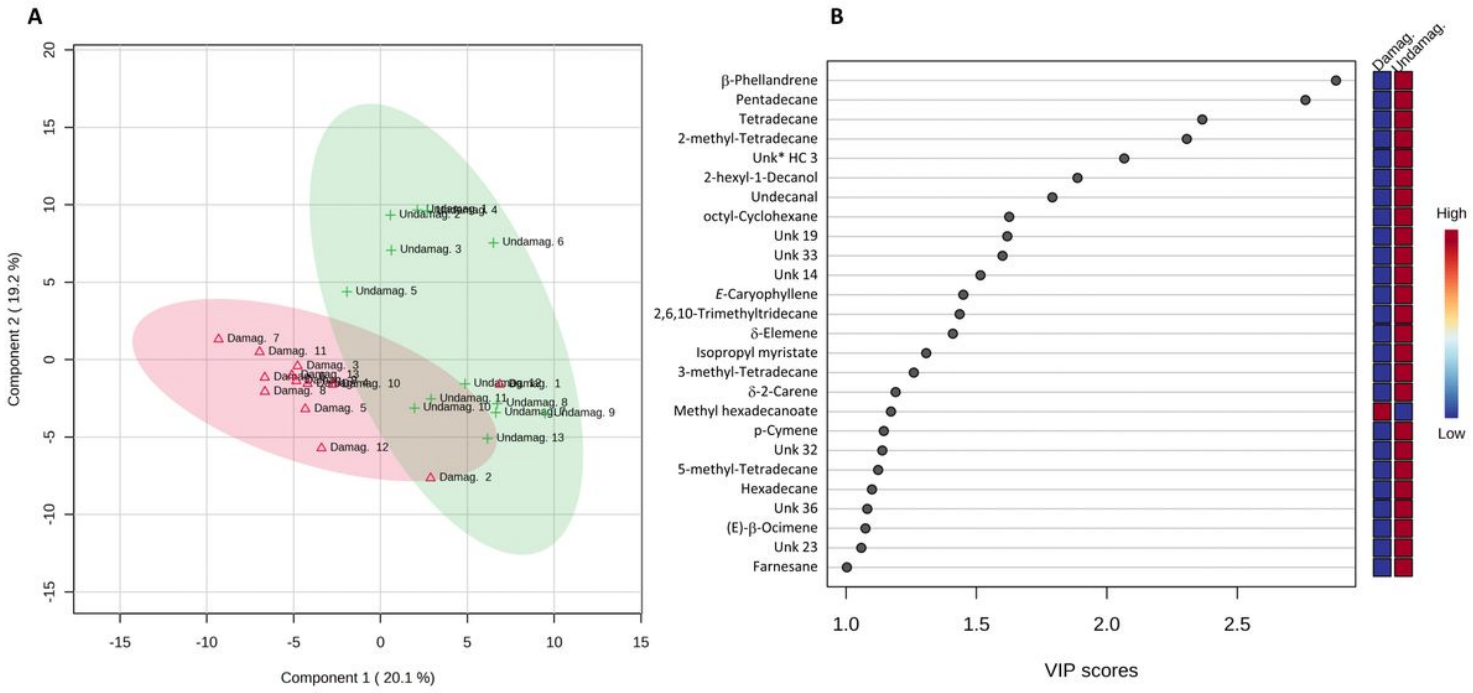

Figure 3

PLS-DA of undamaged vs. *T. vaporariorum*-damaged plants volatiles: Score plot of PC1 vs. PC 2 (the explained variances are shown in brackets, **A**) and VIP score plot showing compound with VIP > 1 (**B**). *Unk: Unknown (in the Case on Unk HC 3, it was classified as hydrocarbon (HC).

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