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Repellency Potential of Tomato Herbivore-Induced Volatiles Against the Greenhouse Whitefly (*Trialeurodes vaporariorum*) (Hemiptera: Aleyrodidae)

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Abstract

The greenhouse whitefly, *Trialeurode vaporariorum*, is among the key pests of tomato (*Solanum lycopersicum*) in sub-Saharan Africa with *Tuta absoluta*, spider mite, thrips, and fruitworms. To understand the interaction between the pest and the plant's herbivory-induced plant volatile (HIPVs), we investigated the repellency of four tomato cultivars (Kilele F1, Assila F1, Red Beauty F1, and Nemonneta F1) upon infestation by *Trialeurode vaporariorum*. We analyzed the behavioral response of *T. vaporariorum* to infested and uninfested tomato plants of these cultivars using olfactory bioassays followed by gas chromatography–mass spectrometry (GC–MS) analyses of emitted volatiles. *Trialeurode vaporariorum* was attracted to uninfested plants of all four tomato cultivars. However, two cultivars Kilele F1 and Red Beauty F1 were no longer attractive to the whitefly when they were already infested by the pest. GC–MS analyses identified 25 compounds, 18 monoterpenes, 3 sesquiterpenes, 2 xylenes, 1 aldehyde, and 1 carboxylic compound in the 4 uninfested and infested cultivars. Based on the insects' behavioral response, 1,8-cineole, *p*-cymene, and limonene did not attract *T. vaporariorum* at varying concentrations when combined with Red Beauty F1, the most attractive tomato cultivar. This repellence behavioral response can be used as a basis for improvement of other vegetable crops for the management of arthropod pests as for odor masking technique.

Graphical Abstract

Healthy tomato
+
Whitefly → 1,8 cineole / E-B-ocimene / Limonene / a-pinene / p-cymene
−
Infested tomato
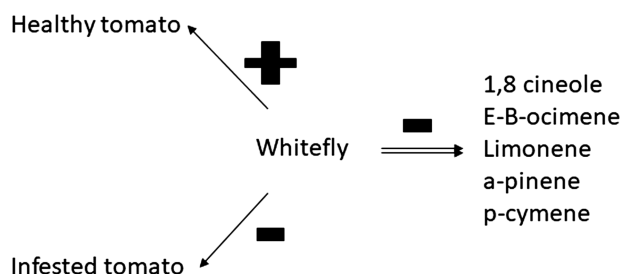
Key words: olfactometer, GC–MS, HIPV, repellent, IPM

Tomato, *Solanum lycopersicon* Mill. (Solanaceae), is the second most produced vegetable crop after potato in Kenya (Mabele and Ndong'a 2019). Tomato production is high across the continent, and Kenya is ranked among the leading tomato-producing countries in Africa with a total production of 540,000+ MT in 2019 (FAO 2020). Tomato fruit is a functional food because of its medicinal

properties (Odame et al. 2008) as well as its vitamins and nutrients including vitamin A, C, phosphorous, magnesium, potassium, and calcium (Frusciante et al. 2007). The tomato fruit is also rich in lycopene and antioxidants that reduce the risk of cancer (Miller et al. 2002). To improve both qualitative and quantitative aspects, over the years, tomato crops have undergone intensive improvement to tackle problems that include quality (flavor, nutritional content), quantity (yields and size), tolerance, to biotic (disease, insects pests, and nematodes), and abiotic stresses (salinity, heat tolerance, and drought; Minja et al. 2011).

Tomato production in Kenya faces many challenges including lack of suitable tomato varieties and cultivars, poor seed quality, unsustainable seed delivery systems, pests and diseases, and the excessive use of pesticides (Ochilo et al. 2019). Some of the common pests that affect tomato production include the tomato leafminer (*Tuta absoluta*), aphids, thrips, whiteflies, cutworms, bollworms, leaf miners, spider mites, and nematodes (Ochilo 2019). The greenhouse whitefly, *Trialeurode vaporariorum* (Westwood) (Hemiptera: Aleyrodidae), is one of the key impediments to tomato production in greenhouses in sub-Saharan Africa (Legg et al. 2003). This pest causes yield losses through direct infestation as well as indirect loses through transmitting the tomato chlorosis crinivirus or the Tomato curly stunt begomovirus (Moodley et al. 2019). Broad-spectrum synthetic insecticides are mainly used to control *T. vaporariorum* (Lapidot et al. 2014), but are hazardous for the environment and nontargets (Gross 2013). Finding safer alternatives to these chemicals for pest management including that of greenhouse whiteflies is thus crucial, and semiochemicals have a potential role to play in an integrated pest management (IPM) approach.

Whitefly are known to exploit semiochemicals to their advantage when locating suitable host plants for the purpose of both feeding and oviposition, and they have been shown use kairomones to make choices between host plants (Saad and Mohamad 2013). This behavior is the result of differences in volatile emissions which vary within the varieties, cultivars and accessions of different plants, including tomato (Bleeker et al. 2009, Darshanee et al. 2017, Tu and Qin 2017). When infested with greenhouse whitefly, *Trialeurodes vaporariorum* or *Bemisia tabaci* (Hemiptera: Aleyrodidae), tomato plants tend to produce blends of volatiles mainly comprising mono and sesquiterpenes, as reported by Ángeles López et al. (2012). These authors also showed that tomatoes infested with *T. vaporariorum* change the emission rate of major constitutive tomato volatile organic compounds (VOCs) with a sharp increase in sesquiterpenes. The change in the emission profile of infested plants leads to an increase in the preference of such plants by the whitefly; Darshanee et al. (2017) reported that greenhouse whitefly preferred infested YG tomato plants over intact plants. In addition to attracting greenhouse whiteflies, herbivore-induced plant volatiles (HIPVs) of tomato have also been shown to play a key role in attracting natural enemies of the whitefly. For example, previous studies have demonstrated that *Nesidiocoris tenuis*, a predator of *B. tabaci*, is attracted to the volatile blends released by *B. tabaci* infested tomato plants (Lins et al. 2014).

The ability of tomato to release a wide range of compounds has been demonstrated in previous studies, showing that tomato releases a blend of diverse volatiles from all plant parts throughout the plant lifetime (Van Schie et al. 2007). This enables the plant to constitute a closed-loop defense system against insect pests by releasing HIPV compounds to attract specific natural enemies of attacking pests and repellent herbivores at the same time, and this response was demonstrated by Pérez-Hedo et al. (2015). This type of defense has thus been proposed as a key management strategy in IPM programs that aim to reduce use of pesticides to improve insect diversity in farming ecosystems (Tilman et al. 2002). The use of synthetic pesticide has been shown to reduce insect populations leading not only to loss of insect diversity but also to a decline in food production due to the elimination of key insect species such as pollinators (Gross 2013).

This aim of the present study was thus to identify key repellent compounds that are released by tomato upon attack by greenhouse whiteflies and that can be used in the management of the pest. We hypothesize that the infested tomatoes plants produce volatiles that can repel greenhouse whiteflies. The specific objective of study was to evaluate the behavioral response of *T. vaporariorum* to four cultivars of infested and uninfected tomato plants and to identify potential repellent compounds that could be used for management of this pest.

Materials and Methods

Plant Production

Tomato plants were used to conduct bioassays. Seeds of four tomato cultivars, Kilele F1 (Syngenta Kenya), Assila F1 (East Africa seed Co. Ltd), Nemonneta F1 (Nirit seeds Ltd), and Red beauty F1 (Amira Kenya Ltd), were sown in planting trays filled with a mixture of red soil and manure (ratio of 3:1 v/v). The seedlings were established in a screen house (length 10 M × width 5 M × height 3 M) in ambient conditions (27 ± 2°C temperature, 65 ± 5% relative humidity) at the International Center of Insect Physiology and Ecology (*icipe*), Duduville, Campus Nairobi, Kenya (1°13′17.9″S 36°53′48.1″E). Three weeks postplanting, the tomato seedlings were transplanted into individual plastic pots (15 cm diameter × 15 cm height).

French bean (*Phaseolus vulgaris*) plants were used to maintain the colony of *T. vaporariorum*. French bean seeds were purchased from Kenya Seeds Company Limited and directly sown in plastic pots of the above-mentioned dimensions using the same soil mix. All the plants were watered three times a week and fertilized with a N:P:K (12:10:8) foliar fertilizer at an application rate of 50 g/20 liter 2 wk after planting. The fertilizer was purchased from local agrochemical stores in Nairobi, Kenya.

Insect Rearing

A colony of *T. vaporariorum* was created at *icipe* using a cohort of insects collected from an insecticide-free experimental field at Kenya Agricultural and Livestock Research Organization (*kalro*), Kimbimbi Research Station, Kirinyaga County (0°37′11.3′S, 37°22′08.0′E) in 2016. The insects were reared in a laboratory maintained at ambient room conditions (25 ± 1°C temperature, 50–60% relative humidity, and 12:12 [L:D] photoperiod). In the laboratory, the insects were kept in Perspex cages (40 cm × 40 cm × 50 cm) and provided with 4-wk-old potted French beans plants for oviposition. Every 2 d, the infested bean plants were removed from the cage and replaced with fresh uninfested ones. Then infested plants were kept in the laboratory. Thereafter, the plants were defoliated and the leaves with the nymphs were placed in similar cages to those used for oviposition, 1–3 d prior to reaching the adult stage.

Tomato Infestation With Greenhouse Whiteflies

A single 4-wk-old (post-transplant) tomato plant was placed in a Perspex cage (40 cm × 40 cm × 50 cm). About 50 female *T. vaporariorum* were introduced in the cage and allowed to oviposit for 72 h under laboratory conditions (25 ± 1°C temperature, 50–60% relative humidity, and 12:12 [L:D] photoperiod). Thereafter, the plant was removed from the cage, and all the adult insects were recovered using an aspirator. The plants were used in the bioassays and to collect volatiles.

Olfactometer Assays With Tomato Plants

The behavioral response of greenhouse whitefly to tomato volatiles was studied using a Y-tube olfactomètre oriented vertically. The Y-tube olfactometer consisted of a Y-shaped glass tube (0.6 cm internal diameter, 10.5 cm arm length, 9.5 cm stem length, and a 60° angle at the junction intersection). Compressed air from an electrical pump (KnF, Laboport, Lagallais, PA Saintes, France) was purified through activated charcoal and regulated by a flow meter (Aalborg, Orangeburg, NY) at 50 ml/min and split into two before being delivered to two 5l Duran glass jars with clean air passing through and into the Y-tube olfactometer arms. Before the experiment, the Duran glass jars were cleaned with distilled water and left to dry. The plastic pots holding the test plants were covered with aluminum foil to avoid odor pollution.

Three different bioassays were conducted using the four tomato cultivars as follows: 1) clean air versus uninfested tomato cultivar, 2) uninfested tomato versus infested tomato (the same cultivar), and 3) uninfested tomato cultivar versus uninfested tomato cultivar (a different cultivar for the purpose of comparison). The Y-tube olfactometer bioassay was conducted in the laboratory at ambient conditions (25 ± 1°C temperature, 50–60% relative humidity) inside a box (20 cm × 20 cm × 30 cm), illuminated from above with an 8W fluorescent lamp from 09:00 to 17:00 h. Before the experiment, the Y-tube was cleaned with 70% ethanol and oven dried. Before the experiment, the greenhouse whiteflies were starved for 2 h before use. A single whitefly was placed in the base of the main arm of the olfactometer and allowed to make a choice for a period of 10 min. The insect was considered to have made a choice when it moved halfway through the Y-tube arm towards either of the odor sources. A no-choice response was recorded when the whitefly made no choice within the 10-min time limit. After five insects were tested, the entire Y-tube setup was rotated 180° to avoid any influence of asymmetries in the setup. Sixty insects were observed in six replicate studies of 10 insects each. The Y-tube was cleaned with 70% ethanol when 10 insects had been tested.

Collection of Volatiles

Four tomato cultivars were used to collect volatiles. Headspace volatiles were simultaneously collected from a pair of tomato plants of the same cultivar of either an uninfested or infested plant. This was replicated four times for each cultivar and infestation status (infested and uninfected). Plants of respective tomato cultivars (healthy or infested) were kept separately in clean 5-liter glass jars fitted with ground glass lid. A charcoal-filtered airstream was pulled over the plant material from the bottom to the top of the jar and over a Porapak Q (50/80) 150/75 mg adsorbent (SUPELCO solutions, Bellefonte, PA), which was held between plugs of glass wool in 4 × 40 mm glass tube. The Porapak Q were precleaned with 5 ml of dichloromethane (DCM; Sigma Aldrich, Gillingham, United Kingdom, purity ≥ 99%) to remove contaminants then dried in a stream of nitrogen. Compressed air from an electrical pump (KnF, Laboport, Lagallais, PA Sainte, France) was purified through activated charcoal and pushed through Nalophan bags at a flow rate of 200 ml/min and pulled out through Porapak Q traps at 150 ml/min for 24 h. The difference in flow rates prevented unfiltered air from entering the system (Webster et al. 2008). The Porapak Q absorbents were eluted with 1-ml DCM into glass vials, then concentrated to 50 µl using a stream of nitrogen gas while on ice. The eluent was analyzed using gas chromatography–mass spectrometry (GC–MS) (Agilent technologies 7890A) or stored at –80°C until analysis.

Analysis of Volatiles

Volatile compounds were analyzed using coupled GC–MS on an Agilent Technologies 7890A GC linked to a 5977 MS equipped with an MSD ChemStation E.02.00.493, Wiley 9th/NIST 2008 MS library and a HP5 MS column (30 cm × 0.25 mm internal diameter × 0.25 µm film thickness; JandW, Folsom, CA) directly coupled with a 5977-mass spectrometer. The GC oven temperature program was set to 35°C for 5 min, ramped at 10°C/min to reach 280°C then held for 5.5 min. The concentrated volatiles in 1-µl aliquots were analyzed in the splitless mode, with helium as a carrier gas programmed at a flow rate of 1.2 ml/min. Spectra were recorded at 70 eV in the electron impact ionization mode with an emission current of 34.6 µA. Compounds were identified using retention time, library mass spectra (NIST11, Wiley 9, Adams and Chemecol), electron ionization spectrum, and Kovats retention indices. The retention indices of the identified compounds were determined using the retention times of *n*-alkane (C8–C23) standards. Identification was performed within a chromatographic window. Quantification of compounds was based on peak area versus concentration of the biphenyl internal standard.

Chemicals

The synthetic compounds and their percentage purity: *p*-cymene 99%, 1,8-cineole 99%, limonene 96%, (*E*)-caryophyllene ≥90%, *α*-phellandrene ≥95%, *β*-pinene 99%, *α*-pinene 98%, terpinolene 90%, sabinene 95%, 3-carene ≥90%, 2-carene 97%, *y*-terpinene ≥97%, myrcene 90%, (*E*)-*β*-ocimene ≥90%, *α*-terpinene ≥96%, and camphene 95% were previous identified from tomato cultivars using GC–MS purchased from Sigma–Aldrich (St Louis, MO; Deletre et al. in press).

Olfactometer Bioassays Using Synthetic Compounds

In total, 16 identified compounds from the four tomato cultivars were used to conduct the two sets of Y tube olfactometer bioassays, one with clean air and another combined with Red Beauty F1 tomato, identified as the most attractive tomato cultivar among the selected four. To test the efficacy of 16 identified synthetic compounds a standard concentration of (0.01, 0.1, and 1%) was chosen with a serial dilution starting with 10 µl of the original synthetic compound. The bioassays were set up as follows: for the 16 synthetic compounds and tomato, 1) 50 µl of each of the 16 synthetic compounds at concentrations (0.01, 0.1, and 1%) versus clean air and 2) Red Beauty F1 + 50 µl of each of the 16 synthetic compounds at concentrations (0.01, 0.1, and 1%) versus Red Beauty F1 + 50 µl dichloromethane solvent. The Y-tube olfactometer bioassay was conducted as described previously for tomato volatiles.

Statistical Analysis

The behavioral response of the greenhouse whitefly to different treatments is expressed as percentage response (%) $[(n/N)*100]$ where N corresponds to the total number of responding greenhouse whiteflies and n is the number of greenhouse whiteflies that responded to a given treatment. Nonrespondent greenhouse whitefly were discarded from the analysis. The frequency count data were subjected to a χ^2 test (χ^2) with Bonferroni correction to test the hypothesis that greenhouse whitefly choice between a pair of odors deviates from the null model of odor source being chosen with equal frequency (Matu et al. 2021). The null hypothesis was that greenhouse whitefly had a 50:50 distribution across the two arms of the olfactometer. A nonparametric Kruskal–Wallis test followed by a Dunn's test

was used to analyze differences in the emission of volatiles between healthy and infested tomato cultivars. All statistical analyses were conducted in R (R Core Team 2018).

Results

Response of the Greenhouse Whitefly to Tomato Cues

Greenhouse whitefly behavioral response was 50.9:49.1 to clean air from the blank 5-liter glass jars connected to either arm of the Y-tube olfactometer, confirming the absence of bias in the orientation of the Y-tube (Fig. 1). The greenhouse whiteflies were attracted to all four tomato cultivars: Assila F1 (χ^2 = 9.931, df = 1, P = 0.001), Red Beauty F1 (χ^2 = 10.286, df = 1, P = 0.001), Nemonneta F1 (χ^2 = 6.333, df = 1, P = 0.011), and Kilele F1 (χ^2 = 5.786, df = 1, P = 0.016) when tested against clean air (Fig. 1a). Uninfested Kilele F1 (χ^2 = 12.519, df = 1, P = 0.001) and Red Beauty F1 (χ^2 = 10.286, df = 1, P = 0.001) tomato cultivars were more attractive to greenhouse whiteflies than their respective whitefly infested tomato cultivars. However, no significant attraction or repellence was observed from uninfested versus infested Nemonneta F1 and Assila F1 (Fig. 1b). The cultivar Red Beauty F1 was significantly attractive to *T. vaporariorum* than Assila F1 (χ^2 = 12.071, df = 1, P = 0.001) and Kilele F1 (χ^2 = 9.931, df = 1, P = 0.001) but not than Nemonneta F1. Assila F1 was notably more attractive than Nemonneta F1 (χ^2 = 11.364, df = 1, P = 0.001; Fig. 1c).

Analysis of Volatile Compounds Emitted by the Tomato Cultivars

In total, 25 compounds were identified in the emissions of the four tomato cultivars. These compounds included 1 carbocyclic compound, 1

aldehyde, 2 xylenes, 18 monoterpenes, and 3 sesquiterpenes (Table 1). When all the compounds emitted by the four tomato cultivars were compared for variance only three compounds showed any significant difference: β-pinene (P = 0.048), p-cymene (P = 0.043), and one unidentified (P = 0.043). Among the 25 known compounds identified, nine were found to have 10-fold variation between healthy and infested plants in at least one of the tomato cultivars, these included o-xylene (uninfested Kilele), sabinene (uninfested Kilele), β-pinene (uninfested Red Beauty, Assila, and Kilele), α-phellandrene (uninfested Red Beauty), 3-carene (uninfested Assila and Kilele), p-cymene (uninfested Assila and Kilele; infested Nemonneta), β-phellandrene (uninfested Red Beauty and infested Nemonneta), y-terpinene (uninfested Assila), and terpinolene (uninfested Assila and Kilele).

Response to Synthetic Compounds Relative to Clean Air

Among synthetic compounds, (E)-β-ocimene, limonene, p-cymene, and α-pinene were repellent to greenhouse whitefly, while 1,8-cineole was attractive when tested against clean air. At a concentration of 0.01%, β-ocimene (χ^2 = 15.291, df = 1, P = 0.001) and limonene (χ^2 = 9.281, df = 1, P = 0.002) elicited a significant repellent response from *T. vaporariorum* relative to clear air. However, at the same concentration, 1,8-cineole (χ^2 = 10.286, df = 1, P = 0.001) was attractive (Fig. 2a). At 0.1% concentration, *T. vaporariorum* was not attracted to p-cymene (χ^2 = 8.6429, df = 1, P = 0.003), β-ocimene (χ^2 = 8.018, df = 1, P = 0.004), and limonene (χ^2 = 4.741, df = 1, P = 0.029), but it was attracted to 1,8-cineole (χ^2 = 4.581, df = 1, P = 0.033; Fig. 2b). At 1% concentration, (E)-β-ocimene (χ^2 = 13.755, df = 1, P = 0.001), α-pinene (χ^2 = 7.692, df = 1, P = 0.005), and p-cymene (χ^2 = 7.407, df = 1, P = 0.006) elicited a repellence behavioral response on
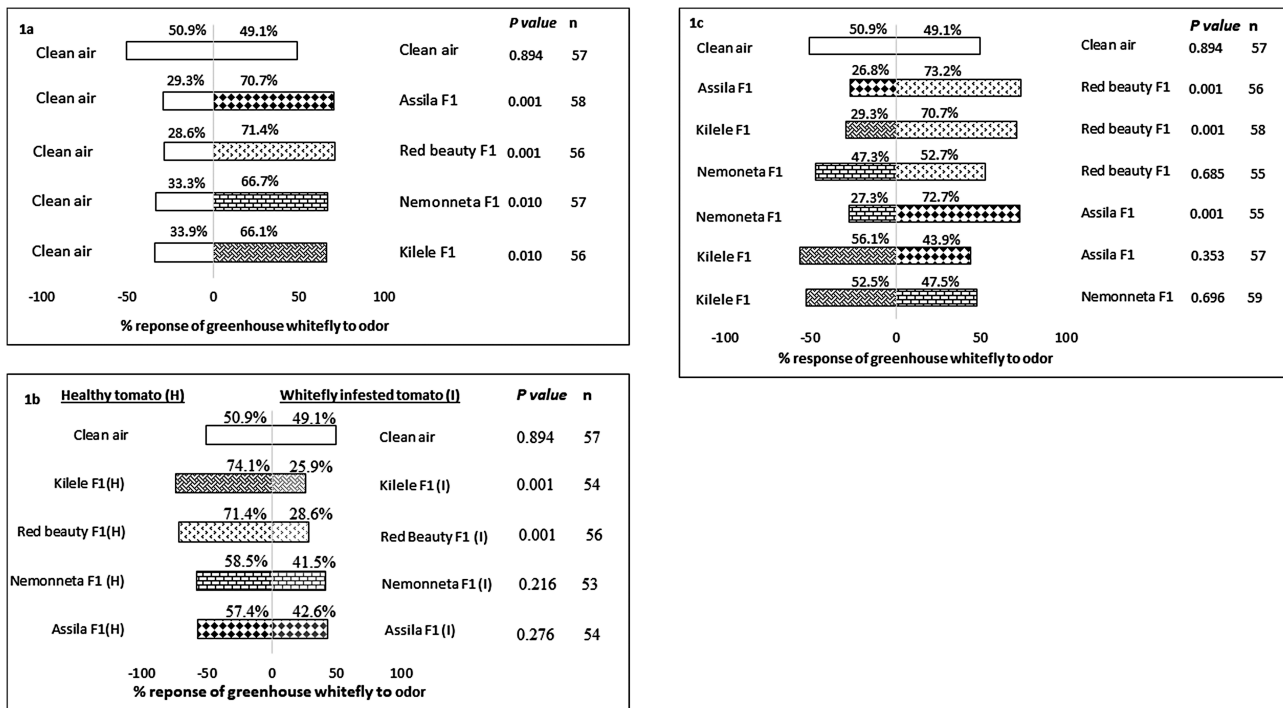
Fig. 1. Percentage response of greenhouse whitefly in a two-choice set up in a Y-tube olfactometer assay: (a) four tomato cultivars (Assila F1, Red Beauty F1, Nemonneta F1, and Kilele F1) were tested individually against clean air (control), (b) four tomato plant cultivars (Red Beauty F1, Assila F1, Nemonneta F1, and Kilele F1) were tested against each other, and (c) healthy tomato cultivars (H) versus whitefly infested plants (I) of Kilele F1, Red Beauty F1, Nemonneta F1, and Assila F1 were compared. p value represents χ^2 goodness-of-fit test; n is the number of responding insects.

Table 1. Mean amount (ng/plant/h) of volatile organic compounds identified in the headspace of healthy plants and whiteflies-infested tomato plants ($n = 4$)

RI	KL	RT	Name	Red Beauty F1		Assila F1		Kilele F1		Nemonneta F1		p-value
				Healthy	Infested	Healthy	Infested	Healthy	Infested	Healthy	Infested	
796	796	6.772	2-Hexanol	0.13 ± 0.06	0.26 ± 0.15	0.21 ± 0.05	0.16 ± 0.02	0.05 ± 0.05	0.03 ± 0.03	0.09 ± 0.05	0.07 ± 0.03	0.141
865	865	8.369	p-Xylene	0.08 ± 0.04	0.18 ± 0.11	0.18 ± 0.13	0.06 ± 0.04	0.44 ± 0.03	0.09 ± 0.02	0.14 ± 0.06	0.09 ± 0.06	0.329
889	888	8.919	o-Xylene	—	—	—	0.03 ± 0.03	0.12 ± 0.05	0.01 ± 0.01	0.06 ± 0.04	0.03 ± 0.03	0.483
936	939	9.838	α-Pinene	3.14 ± 1.60	8.14 ± 3.45	1.11 ± 0.34	0.68 ± 0.39	3.66 ± 1.98	0.74 ± 0.26	3.61 ± 1.53	2.39 ± 1.09	0.102
946	946	10.101	Camphene	—	—	—	—	0.23 ± 0.23	—	0.50 ± 0.50	—	0.850
973	972	10.154	3,7,7-Trimethyl-1,3,5-cycloheptatriene	3.92 ± 3.27	—	0.45 ± 0.18	0.42 ± 0.29	1.65 ± 1.18	0.51 ± 0.23	2.67 ± 1.25	2.33 ± 1.16	0.253
977	977	10.247	Sabinene	0.19 ± 0.16	1.13 ± 0.66	0.16 ± 0.07	0.03 ± 0.03	0.32 ± 0.20	0.02 ± 0.02	1.25 ± 1.13	0.60 ± 0.37	0.508
978	978	10.323	β-Pinene	0.29 ± 0.15ab	2.55 ± 0.89b	0.34 ± 0.19ab	0.03 ± 0.03a	0.60 ± 0.39ab	0.02 ± 0.02a	1.69 ± 1.54ab	1.28 ± 0.80b	0.048
993	992	10.622	Myrcene	0.67 ± 0.59	0.89 ± 0.33	0.69 ± 0.22	0.23 ± 0.17	0.83 ± 0.27	0.25 ± 0.15	3.16 ± 2.64	1.84 ± 0.88	0.115
1,002	1,001	10.686	2-Carene	20.88 ± 16.56	—	7.67 ± 2.51	3.99 ± 2.19	18.93 ± 9.83	4.34 ± 1.71	26.12 ± 10.00	8.73 ± 6.52	0.500
1,006	1,006	10.739	α-Phellandrene	4.83 ± 3.89	0.10 ± 0.10	1.24 ± 0.47	0.81 ± 0.55	3.37 ± 1.86	0.93 ± 0.37	4.64 ± 1.66	1.78 ± 1.30	0.118
1,011	1,011	11.055	3-Carene	0.26 ± 0.21	1.08 ± 0.41	3.29 ± 1.99	0.24 ± 0.14	4.36 ± 2.57	0.17 ± 0.06	9.95 ± 9.00	10.93 ± 7.58	0.275
1,016	1,017	11.236	α-Terpinene	2.42 ± 2.08	—	0.58 ± 0.25	0.35 ± 0.23	1.29 ± 0.67	0.36 ± 0.18	1.61 ± 0.71	1.56 ± 0.58	0.228
1,028	1,027	11.294	p-Cymene	—	1.03 ± 0.42ab	2.86 ± 1.70a	0.14 ± 0.09a	3.39 ± 1.74b	0.09 ± 0.06b	0.23 ± 0.17a	9.17 ± 9.06ab	0.043
1,030	1,030	11.423	β-Phellandrene	46.57 ± 35.10	0.10 ± 0.10	21.29 ± 8.23	12.19 ± 6.61	55.68 ± 29.00	14.49 ± 5.07	56.24 ± 16.14	4.88 ± 3.41	0.066
1,032	1,032	11.54	Limonene	—	19.14 ± 10.05	—	—	—	—	—	14.31 ± 14.31	0.442
1,034	1,035	11.681	1,8-Cineole	—	—	—	—	—	—	0.20 ± 0.20	5.86 ± 5.78	0.508
1,042	1,041	11.768	(Z)-β-Ocimene	—	—	—	0.18 ± 0.18	0.47 ± 0.28	—	0.82 ± 0.82	1.89 ± 1.42	0.616
		11.78	unidentified 1	1.13 ± 0.59a	—	0.03 ± 0.03b	—	0.41 ± 0.24ab	0.08 ± 0.05ab	0.85 ± 0.29a	0.15 ± 0.12ab	0.043
1,051	1,051	11.821	(E)-β-Ocimene	0.35 ± 0.35	0.19 ± 0.19	0.71 ± 0.51	0.22 ± 0.22	0.83 ± 0.45	0.11 ± 0.11	1.59 ± 1.33	3.25 ± 2.18	0.393
1,061	1,060	11.926	γ-Terpinene	1.26 ± 1.08	—	1.32 ± 0.90	0.12 ± 0.12	1.11 ± 0.71	0.24 ± 0.24	0.51 ± 0.51	2.60 ± 1.74	0.794
1,092	1,090	12.119	Terpinolene	0.13 ± 0.08	—	0.58 ± 0.26	0.03 ± 0.03	0.68 ± 0.49	0.06 ± 0.06	1.58 ± 1.26	1.86 ± 1.03	0.313
		12.833	Unidentified 2	—	—	—	—	—	—	—	0.09 ± 0.09	—
1,125	1,128	13.16	Allo-Ocimene	—	—	—	—	—	—	—	0.03 ± 0.03	—
1,402	1,400	13.342	β-Elemene	0.17 ± 0.15	—	—	—	—	—	—	0.05 ± 0.05	0.741
1,432	1,428	16.665	(E)-Caryophyllene	2.14 ± 1.93	0.72 ± 0.43	0.08 ± 0.08	0.14 ± 0.14	2.00 ± 0.71	0.36 ± 0.28	0.70 ± 0.52	1.65 ± 1.30	0.369
1,465	1,460	17.817	α-Humulene	0.65 ± 0.57	—	0.02 ± 0.02	0.10 ± 0.10	0.44 ± 0.23	0.18 ± 0.12	0.70 ± 0.52	1.86 ± 1.50	0.771

—, not detected; RI, retention indices calculated relative to n-alkane c8-c30 in the hp-5ms column; KL, Kovat's retention indices from the literature; RT, retention time in minutes; Name, identification of compounds based on the retention time, retention indices and mass spectra using libraries, i.e. NIST and Wiley, Adams, and Chemecol, comparison with published mass spectra and retention indices from online NIST library and confirmation with authentic standards; p value of the nonparametric Kruskal–Wallis ANOVA for comparison of volatile compounds between healthy and *T. absoluta*-infested plants.

Significant values are in bold, means within rows followed by different letters are significantly different.

T. vaporariorum. None of the compounds elicited attractive responses to *T. vaporariorum* at the same concentration (Fig. 2c).

Response to Synthetic Compounds Relative to Cultivar Red Beauty F1 Volatiles

1,8-Cineole, *p*-cymene, and limonene reduced the attractiveness of tomato to *T. vaporariorum*. The response of whiteflies varied with the concentration. At 0.01%, *T. vaporariorum* was repelled by the tomato and 1,8-cineole combination ($\chi^2 = 11.792$, df = 1, $P = 0.001$; Fig. 3a). At 0.1% concentration, *T. vaporariorum* was repelled by 1,8-cineole ($\chi^2 = 15.077$ df = 1, $P = 0.001$) and *p*-cymene ($\chi^2 = 9.618$, df = 1, $P = 0.001$; Fig. 3b). At 1% concentration, *T. vaporariorum* was repelled by *p*-cymene ($\chi^2 = 9.981$, df = 1, $P = 0.001$), 1,8-cineole ($\chi^2 = 7.043$, df = 1, $P = 0.007$), and limonene ($\chi^2 = 6.48$, df = 1, $P = 0.010$; Fig. 3c).

Discussion

Herbivorous insects are attracted to their host plant via a variety of cues. In the present study, we hypothesized that the four tested tomato cultivars were attractive to greenhouse whiteflies and that they showed different degrees of attractiveness to the insects. Our findings confirmed this hypothesis, as all the tomato cultivars we tested

elicited a positive response from the whiteflies. However, the degree of attractiveness varied with cultivar and was highest for Red Beauty F1 compared with clean air. This was attributed to the fact that the composition of volatile emitted by the tomato accession changes in terms of quantity and quality of volatiles. But we also noticed years of breeding was negatively correlated to whitefly response, which has mainly focused on improving tomato quality and quantity to the detriment of volatile composition (Simmons and Gurr 2005).

The process of human-mediated plant selection has improved agronomic traits such as organoleptic properties and yield, but altered how cultivated plants interact with their immediate environment compared with wild plants (Milla et al. 2015). Whitehead et al. (2017) have evidenced an increase in pest attacks on domesticated plants compared to their wild counterparts. These authors argued that domestication of plants did not have a consistent effect on the specific plant defense trait 'resistance' whose underlying markers include secondary metabolites and physical barriers. In our study, when the four tomato cultivars were compared with one another, Red Beauty F1 was found to attract greenhouse whiteflies more than Assila F1 and Kilele F1, and Assila F1 was more attractive to whitefly than Nemonneta F1.

Infestation of tomato cultivars by whiteflies differentially affected their attractiveness to the insects when tested against their respective healthy counterparts, with Kilele F1 and Red Beauty F1 showing
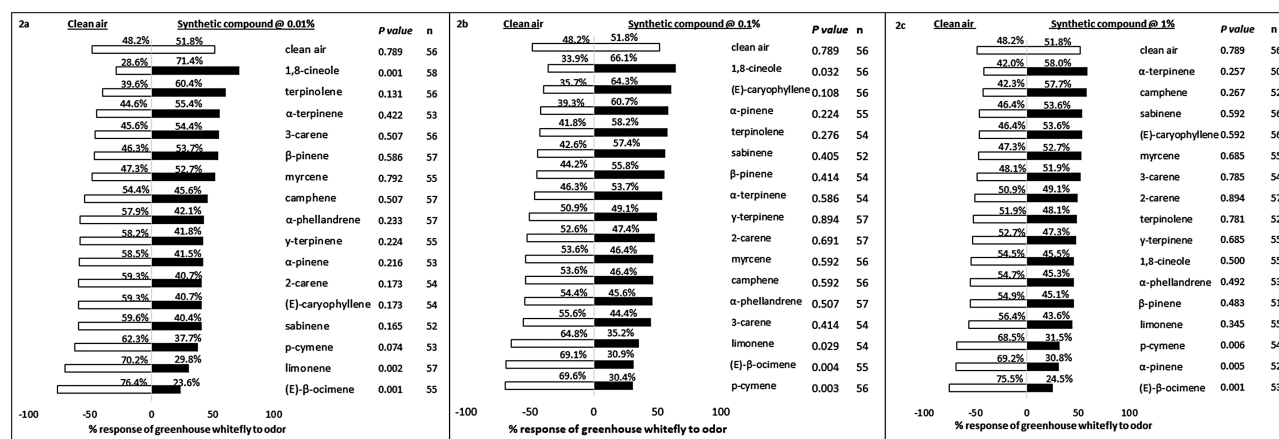

Fig. 2. Percentage response of greenhouse whitefly to clean air versus synthetic compounds tested at concentrations of (a) 0.01%, (b) 0.1%, (c) 1% in a Y-tube olfactometer bio-assay. *p*-value represents χ^2 goodness-of-fit test; *n* is the number of responding insects.
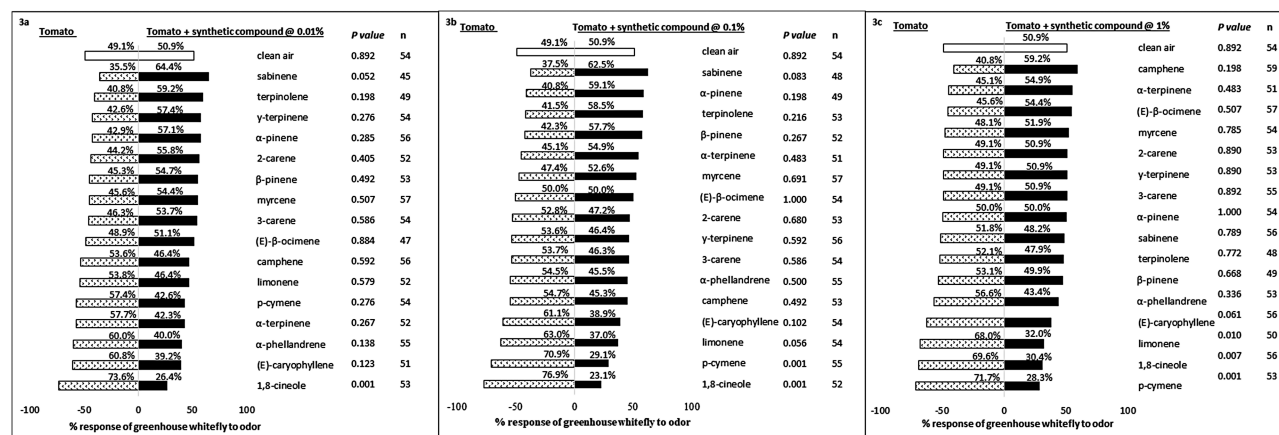

Fig. 3. Percentage response of greenhouse whitefly to Red Beauty F1 tomato versus Red Beauty F1 tomato + 50 µl of synthetic compound tested at (a) 0.01%, (b) 0.1%, (c) 1% concentration in a Y-tube olfactometer bio-assay. *p* value represents χ^2 goodness-of-fit test; *n* is the number of responding insects.

significant less attraction than their healthy counterparts. In a similar study, Darshanee et al. (2017) found that the greenhouse whitefly was more attracted to healthy YG tomato cultivars than to their infested counterparts. The differential response of the whitefly to infested and healthy tomato plants could be explained by the fact that the insects perceived the infested plant through chemical cue as poor sources for the development of their offspring and high competition. Indeed, the greenhouse whitefly has been demonstrated to activate salicylic acid-mediated response for HIPV synthesis (Kempema et al. 2007), which could be used as repellents against whitefly. Infestation of greenhouse tomato by whitefly can alter the composition of volatile emissions, thereby creating a difference between infested and healthy plants. This change in volatile profile was reported in tomatoes infested with the greenhouse whitefly by Ángeles López et al. (2012) and in lima bean (*Phaseolus lunatus*) by Birkett et al. (2003).

Repellents produced by tomato plants in the form of HIPVs have been shown to play a role in the management of pests including the greenhouse whitefly (Frost et al. 2008). In our study, the greenhouse whiteflies were found to be repelled by different concentrations of limonene, *p*-cymene, and (*E*)-*β*-ocimene in control trials. In previous studies, limonene has been shown to be repellent against other pests. For example, Li et al. (2014) reported that limonene reduced egg-laying in *B. tabaci* by more than 80% in a greenhouse experiment. The greenhouse whitefly has also been found to be repelled by linalool and *β*-ocimene in several studies making these compounds good candidates for use in 'push–pull' management of this pest (Tu and Qin 2017). Greenhouse whitefly attraction to VOCs has also been previously exploited as an alternative to the use of synthetic pesticides (Gorski 2004). We found that 1,8-cineole was attractive to greenhouse whitefly at low concentration and when the alternative was clean air rather than a tomato plant. The same compound was also documented to attract the *B. tabaci* B-biotype (e.g., Cao et al. 2008). However, the behavioral response of an insect depends on the concentration (low or high) and the context (host or non host; Deletre et al. 2016). Indeed, an attractant compound can become repellent at high concentration or be a masking odor in the host presence.

Synthetic compounds have been used to mimic HIPVs in several studies that investigated the behavioral response of different insects in an attempt to identify with more environmentally friendly pest management solutions (Norin 2007). In our work, we studied 16 compounds to ascertain their behavioral effect on greenhouse whitefly, Red Beauty F1 being the most attractive cultivar. 1,8-Cineole, *p*-cymene, and limonene elicited a significant repellence behavioral response in greenhouse whitefly. Odor masking techniques have previously been studied as potential alternatives for the management of whitefly, e.g., in the case of sweetpotato whitefly, *B. tabaci*, in which it was shown that applying sesquiterpenes R-curcumene and 7-episingiberene on tomato repelled adult whitefly prior to landing (Bleeker et al. 2011). The same behavioral response of whitefly to synthetic compounds was demonstrated in our study using 1,8-cineole, *p*-cymene, and limonene (repellents) providing insight into volatile compounds that have a 'push' effect. This kind of repellent is called maskant repellent because a masking odor interferes with the host detection/localization, decreases the attractiveness of the host or can also decrease the host attractiveness by changing the host chemical, thereby impeding an insect's host-seeking activity as with HIPVs (Deletre et al. 2016). Repellence of whitefly from host plants using synthetic VOCs has also been reported in tomato crops, where a mixture of D-limonene, citral, and olive oil at a ratio of 63:7:30 was found to repel *B. tabaci* settling on tomato (Du et al. 2016). Production of monoterpenes such as *p*-cymene varies among tomato

varieties and cultivars, but the repellence capability of *p*-cymene was documented in a study in which *B. tabaci* were repelled by *Solanum pennelli* plant treated with *p*-cymene (Bleeker et al. 2009).

In the present study, among the three compounds with some level of repellence against greenhouse whitefly, 1,8-cineole was the most potent compound in terms of repellency. The repellency of this compound has been demonstrated for several other whitefly species. For instance, Sacchetti et al. (2015) reported emissions of VOCs by bottlebrush, *Callistemon phoeniceus* and *C. laevis* (Myrtaceae), in which 1,8-cineole was found to repel sweetpotato whitefly, *B. tabaci* biotype B, in a Y-tube olfactometer bioassay. Repellency by 1,8-cineole has also been reported against other insect pests, including the grain beetle, when 1,8-cineole was shown to strongly repel *Sitophilus granarius* and *S. zeamais* (Curculionidae) while moderate repellence was recorded against *Tribolium castanum* (Tenebrionidae) and *Prostephanus truncates* (Horn) (Bostrichidae) (Obeng-Ofori et al. 1997).

In summary, the results of the present study showed that four tomato cultivars, i.e., healthy Kilele F1, Assila F1, Red Beauty F1, and Nemonneta F1 attract greenhouse whitefly, but upon infestation by the same pest, both Kilele F1 and Red Beauty F1 plants remain significantly attractive to greenhouse whitefly. This is evidence that infested plants can change their volatile compound profile. This change in profile was observed in all the four tomato cultivars with the emission of some compounds declining after infestation by greenhouse whitefly. The compounds capable of repellence observed in this study were 1,8-cineole, *p*-cymene, and limonene when used in synthetic form at different concentrations, with 1,8-cineole standing out as a potential candidate for management of this pest. Our results now need to be confirmed in field conditions before the compounds are commercially rolled out as repellent.

Acknowledgments

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