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The Role of Specific Tomato Volatiles in Tomato-Whitefly Interaction^{1[W][OA]}

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Bemisia tabaci (whitefly) infestations and the subsequent transfer of viruses are the cause of severe losses in crop production and horticultural practice. To improve biological control of *B. tabaci*, we investigated repellent properties of plant-produced semiochemicals. The mix of headspace volatiles, collected from naturally repellent wild tomato accessions, influenced *B. tabaci* initial choice behavior, indicating a role for plant semiochemicals in locating host plants. A collection of wild tomato accessions and introgression lines (*Solanum pennellii* LA716 × *Solanum lycopersicum* 'Moneyberg') were extensively screened for attractiveness to *B. tabaci*, and their headspace profiles were determined by means of gas chromatography-mass spectrometry. Correlation analysis revealed that several terpenoids were putatively involved in tomato-whitefly interactions. Several of these candidate compounds conferred repellence to otherwise attractive tomato plants when applied to the plant's branches on paper cards. The sesquiterpenes zingiberene and curcumene and the monoterpenes *p*-cymene, α -terpinene, and α -phellandrene had the strongest effects in free-choice bioassays. These terpenes also elicited a response of receptors on the insect's antennae as determined by electroantennography. Conversely, the monoterpene β -myrcene showed no activity in both assays. *B. tabaci* apparently uses, besides visual cues, specific plant volatile cues for the initial selection of a host. Altering whitefly choice behavior by manipulation of the terpenoid composition of the host headspace may therefore be feasible.

During the last decades, a worldwide spread of the pest insects *Bemisia tabaci* (Gennadius) and greenhouse whitefly (*Trialeurodes vaporariorum*) has led to local devastation of vegetable and ornamental crops, resulting in large economic losses. The damage whiteflies cause by their feeding behavior, such as affected biochemistry and development (for review, see Inbar and Gerling, 2008), is far exceeded by the secondary, indirect crop losses due to virus transmission. Specifically *B. tabaci* outbreaks are associated with the emergence of viruses for which they serve as vectors (Polston and Anderson, 1997). *B. tabaci* is capable of transmitting >100 different virus species of which the majority belong to the genus *Begomovirus*, such as *Tomato yellow leaf curl virus*, *Tomato mottle virus* (Jones, 2003), and *African cassava mosaic virus* (Maruthi et al., 2001). Damage caused by virus infection ranges from

mild symptoms, such as leaf discolorations, to overall yield reduction, severe fruit necrosis, flower and fruit abortions, and plant death. Viral diseases are particularly severe since no chemical control is available and good sources of virus resistance for interspecific crossing are not always available (Maruthi et al., 2003). To date, only a limited number of virus resistance genes have been identified, and due to high mutation rates, viruses rapidly evolve (Drake and Holland, 1999; García-Andrés et al., 2006) and break monogenic resistances. Herbivores, such as whiteflies and thrips, can apparently benefit from transmitting viruses (Medeiros et al., 2004; Jiu et al., 2007; Belliure et al., 2008).

B. tabaci was originally restricted to subtropical regions and greenhouses. However, the new and extremely invasive B and Q biotypes have the ability to rapidly adapt to more temperate zones and new host species (Jones, 2003; Wan et al., 2008). To date, the main control strategy for many crops is the application of insecticides, though effective spraying is complicated because of the insect's preference for the abaxial side of the leaf (Simmons, 1994). Moreover, *B. tabaci* is difficult to control chemically due to emerging resistance to active ingredients (Horowitz et al., 2005). A new biological control agent, the phytoseiid predator *Typhlodromips swirskii*, has only been successful on plants without trichomes in closed greenhouses (Nomikou et al., 2002). The root-knot nematode resis-

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tance gene *Mi1.2*, which confers partial resistance to *B. tabaci* (Nombela et al., 2003), is widely used in modern tomato (*Solanum* spp.) varieties but is not sufficient to provide adequate protection against whitefly infestations.

During insect host selection, orientation, and landing, both visual and olfactory cues play a predominant role (Visser, 1988). Color is an important factor in host-plant selection, and it was shown that *B. tabaci* reacts to blue-UV and yellow wavelengths (Van Lenteren and Noldus, 1990). The olfactory stimuli associated with the host plant initiate host targeting, whereas visual cues improve the accuracy of landing. In the initial phase of host targeting, olfaction may cause a positive chemotactic response, i.e. a flight up an odor gradient. Plant odor specificity might be achieved by a particular ratio of constituent volatiles (Bruce et al., 2005a). In the case of whiteflies, the role of olfaction in attraction or repellence has not received much prior attention. After host contact, *B. tabaci* evaluates host plant quality by labial dabbing and probing using piercing mouthparts. By probing, persistent viruses are transmitted via the insects' salivary glands and mouthparts (Ghanim et al., 1998; Rosell et al., 1999). Therefore, to avoid virus transmission by *B. tabaci*, probing should be prevented.

Volatile organic compounds released by plants can act as semiochemicals. They play an important role in enabling insects to recognize host plants from a distance (Schütz et al., 1997; Bruce et al., 2005a) or in attracting predators and parasitoids upon herbivory (De Moraes et al., 1998; Van Poecke and Dicke, 2002; Kappers et al., 2005). Moreover, they can play a role in the direct defense against herbivores and pathogens (Kessler and Baldwin, 2001; Shiojiri et al., 2006). A large number of different plant volatiles, with numerous ecological roles, have been identified so far (Sacchettini and Poulter, 1997; Pichersky et al., 2006). The largest class of plant volatiles is derived from the isoprenoid or terpenoid pathway. Solanaceous plants, like tomato, often make use of these terpenes for the defense against herbivores (Snyder et al., 1993; Kennedy, 2003). Some terpenes have been shown to exhibit repellent properties to insects (Peterson et al., 2002; Birkett et al., 2004; Terry et al., 2007). These plant-produced semiochemicals can potentially be used as insect repellents of natural origin, thus providing an alternative to the use of pesticides (Peterson and Coats, 2001). Engineering terpene emission to make crop plants more attractive to herbivore enemies has already been shown to be feasible (Degenhardt et al., 2003; Kappers et al., 2005; Schnee et al., 2006).

The aim of this study is to identify the role of plant volatiles in the *B. tabaci*-tomato host interaction and to identify the terpenes that cause repellence of a selection of wild tomato accessions. The potential of several terpenes as repellent olfactory cues in *B. tabaci* host-preference behavior has been assessed in behavioral studies and through electroantennography (EAG).

RESULTS

Characterization of *B. tabaci* Populations

Morphologically indistinguishable *B. tabaci* populations are known to exhibit large variations in biological traits as well as a distinct genetic variation. *B. tabaci* populations were collected from crop production sites all over the world, and the amplified fragment length polymorphism (AFLP) technique was used to investigate the extent and nature of intraspecific variability of the assorted *B. tabaci* populations.

Genetic diversity assessment of the various populations revealed three distinct clusters, which appear to be geographically correlated (Supplemental Fig. S1) and could be identified as biotype Q, biotype B, and an undetermined (U) biotype using biotype-specific PCR markers (Khasdan et al., 2005). Apparently, parts of Asia are dominated by biotype U and parts of Southern Europe by biotype Q. Biotype B dominates the greenhouses of several countries spread around the world. Genetic variation was not correlated to the seven crop species from which the *B. tabaci* populations were collected (data not shown), as expected for a generalist herbivore.

Tomato Semiochemical Cues and *B. tabaci* Preference Behavior

The relative attraction of *B. tabaci* to the accessions of the tomato collection, containing 16 wild and five cultivated tomatoes, was determined in free-choice bioassays with *B. tabaci*. In these bioassays, the number of flies per plant was counted 10 min after release in the center of the arena. Examination of host-preference behavior of two selected biotype populations (B and Q) revealed no differential preference behavior of these whiteflies in free-choice bioassays with cultivated and wild tomato (Fig. 1A; $P > 0.5$). Therefore, it was decided to use the Q biotype (Almería B) population in all further assays.

Prior to starting large-scale bioassays, it was established that *B. tabaci* preference behavior is indeed influenced by the volatiles in the headspace of the tomato plants. Preference assays were done with four different *Solanum lycopersicum* cultivars of the same age, height, shape, and color. Afterward, the experiment was repeated with the same plants now individually placed under a mesh cover (Supplemental Fig. S2) to conceal the shape and color of the plant so that whiteflies could make a choice based on the volatiles they detect. Even though fewer whiteflies were recaptured in the absence of visual cues, the relative distribution of whiteflies over tomatoes with different attraction did not change ($P > 0.5$) compared to that obtained with exposed plants (Fig. 1B). The presence of the *Mi1.2* gene in two of the cultivated tomatoes (Motelle and Mogeor) had no influence on the choice of the whiteflies in our experimental setup.

Sequential stepwise bioassays revealed a clear preference of the whiteflies for cultivated tomato plants

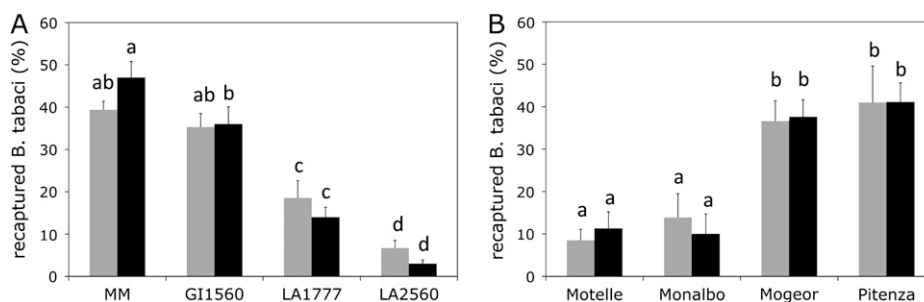


Figure 1. Relative preference (as percentage recaptured *B. tabaci*, $n = 200$) for different tomato varieties. A, Results for genetically different *B. tabaci* populations; biotype Q (gray bars) and biotype B (black bars) on *S. lycopersicum* 'Moneymaker' (MM), *S. habrochaites* GI1560, *S. habrochaites* LA1777, and *S. pennellii* LA2560. B, Results for *B. tabaci* biotype Q in the presence (gray bars) and absence (black bars) of visual cues in a setup with four *S. lycopersicum* plants: cv Motelle (containing *Mi1.2*), cv Monalbo, cv Mogeor (containing *Mi1.2*), and cv Pitenza. Bars represent the means of three experiments (\pm SE). Significant differences ($P < 0.05$) are indicated by different letters.

over the wild tomato accessions. *Solanum pennellii* LA2560 and *Solanum habrochaites* PI127826 ranked as least preferred, whereas *Solanum peruvianum* LA1708 was only slightly less attractive than the cultivated tomato plants as indicated by the classification in seven preference groups (Table I). Next, we determined whether the collected headspace volatiles of the two least preferred tomatoes (*S. pennellii* LA2560 and *S. habrochaites* PI127826) could reduce the attractiveness of the cultivated tomato (*S. lycopersicum* 'Moneymaker'). The headspace volatiles collected over 24 h were applied to a *S. lycopersicum* 'Moneymaker' plant on 10 filter paper cards distributed over the entire plant (for concentrations, see Table I). Free-choice

bioassays were performed with four plants, of which one received wild tomato volatiles. In this setup, the cultivated tomato with the headspace volatiles of LA2560 or PI127826 attracted up to 60% less whiteflies (Fig. 2, A and B, respectively). The carrier of the headspace volatiles, pentane-diethylether, alone did not affect the choice of *B. tabaci* (Fig. 2).

Determining Headspace Components as Cues for Repellence

In addition to bioassays, a headspace volatile fingerprint for each tomato plant of our collection was assembled using gas chromatography-mass spectrom-

Table I. Wild tomato accessions (*S. pennellii*, *S. habrochaites*, and *S. peruvianum*) and cultivated tomato lines (*S. lycopersicum*) ranked based on relative preference of *B. tabaci*

1 = Lowest preference; 7 = highest preference. Quantities of candidate semiochemicals ($\mu\text{g } 24 \text{ h}^{-1} 10 \text{ g}^{-1}$ fresh weight) emitted by wild tomato and cultivated tomato, and caryophyllene, which is present in all accessions. Values indicate means (\pm SE; $n = 6$). –, Not detected.

Tomato	Accession	Ranking	β -Myrcene	<i>p</i> -Cymene	γ -Terpinene	Curcumene	Zingiberene	Caryophyllene
<i>S. pennellii</i>	LA716	2	–	0.34 ± 0.13	4.01 ± 1.71	10.83 ± 8.29	68.5 ± 58.9	0.55 ± 0.20
	LA1340	2	1.46 ± 0.45	0.35 ± 0.11	2.49 ± 0.96	4.46 ± 4.35	6.56 ± 6.56	2.01 ± 0.82
	LA2560	1	1.36 ± 0.61	0.47 ± 0.18	5.88 ± 2.50	3.64 ± 3.09	8.44 ± 4.36	3.06 ± 1.50
<i>S. habrochaites</i> (f. <i>typicum</i>)	LA1777	3	0.12 ± 0.10	0.05 ± 0.04	0.06 ± 0.07	12.3 ± 10.8	9.71 ± 9.14	7.39 ± 3.36
	PI127826	1	–	–	0.24 ± 0.24	282.4 ± 116.2	521.0 ± 323.6	0.28 ± 0.18
	PI127827	3	0.01 ± 0.01	0.03 ± 0.03	–	–	–	0.34 ± 0.21
<i>S. habrochaites</i> (f. <i>glabratum</i>)	LA1353	3	0.20 ± 0.12	0.01 ± 0.01	–	–	–	2.56 ± 0.84
	PI126449	4	–	–	0.22 ± 0.22	–	–	8.34 ± 2.61
	PI134417	3	–	–	–	–	–	13.41 ± 5.98
	PI134418	4	–	–	–	–	–	3.08 ± 1.10
	PI251304	3	–	–	–	0.21 ± 0.21	–	7.23 ± 2.81
	IVT701631	4	–	–	–	3.35 ± 3.21	–	7.27 ± 6.31
	LA407	5	–	–	–	0.96 ± 0.96	–	3.64 ± 0.92
<i>S. lycopersicum</i>	GI1560	3	–	–	–	11.1 ± 6.6	3.76 ± 3.76	3.48 ± 0.57
	LA1840	5	0.02 ± 0.02	0.01 ± 0.01	0.05 ± 0.05	0.08 ± 0.05	0.88 ± 0.88	0.12 ± 0.05
	LA1708	5	0.09 ± 0.09	0.07 ± 0.03	1.13 ± 0.68	0.54 ± 0.54	–	2.88 ± 0.86
	Motelle	6	0.08 ± 0.06	0.02 ± 0.01	–	–	–	0.54 ± 0.17
<i>S. lycopersicum</i>	Mogeor	6	0.08 ± 0.05	0.06 ± 0.05	–	–	0.38 ± 0.38	2.24 ± 0.91
	Monalbo	6	0.42 ± 0.15	0.10 ± 0.05	0.60 ± 0.22	–	0.51 ± 0.34	1.37 ± 0.11
	Moneymaker	7	0.07 ± 0.07	0.01 ± 0.01	0.07 ± 0.07	0.11 ± 0.11	–	3.35 ± 2.41
	Pitenza	6	0.17 ± 0.10	0.07 ± 0.04	0.07 ± 0.07	–	–	1.11 ± 0.21

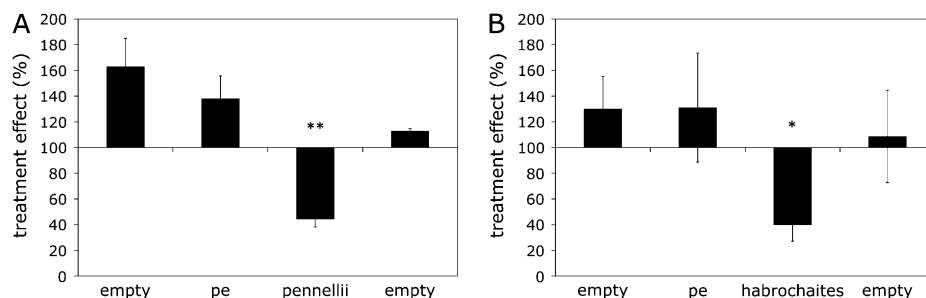


Figure 2. Headspace volatiles from *S. pennellii* (A) and *S. habrochaites* (B) can repel whiteflies. Treatment effect of added wild tomato headspace volatiles in a setup of four *S. lycopersicum* ‘Moneymaker’ plants (expressed as percentage of untreated setup, $n = 200$) on *B. tabaci* biotype Q choice behavior. Empty, Empty carriers; pe, carriers containing pentane:diethylether; pennellii, carriers containing total headspace (collected over 24 h) of *S. pennellii* (LA2560); habrochaites, carriers containing total headspace (collected over 24 h) of *S. habrochaites* f. *typicum* (PI127826). Bars present averages of three experiments (\pm SE). Level of significance: *, $P < 0.05$; **, $P < 0.01$.

etry analyses. In total, 51 major components were identified in the headspace of undamaged plants (Supplemental Table S1). These data, in combination with the preference behavior of the whiteflies, were subjected to multiple linear regression and multivariate ANOVA analyses, which resulted in the identification of five semiochemicals that could influence the choice of *B. tabaci*. An association with reduced preference by the wild tomato accessions was found for the sesquiterpenes zingiberene and curcumene, mostly present in *S. habrochaites* (formerly f. *typicum*) and three monoterpenes from *S. pennellii*: *p*-cymene, γ -terpinene, and β -myrcene. The concentrations of these components produced throughout the complete tomato collection are shown in Table I. However, some compounds are present throughout the whole collection of plants (e.g. caryophyllene). Ketones were present mostly in the *S. habrochaites* glabratum tomatoes, and in this experimental setup, they were not associated to reduced preference.

Because *S. pennellii* accessions appeared to be unattractive to whiteflies, introgression lines of a *S. pennellii* LA716 \times *S. lycopersicum* ‘Moneyberg’ cross were subsequently screened in free-choice bioassays (Supplemental Fig. S3) with the aim to find additional candidate volatiles. None of the introgression lines ranked as repellent as the LA716 parent. Ten introgression lines with lowered attraction and one line with equal whitefly attraction compared to the Moneyberg parent were selected, and the headspace composition of these selected lines and the Moneyberg parent was determined. Seven monoterpenes were detected in the headspaces of the parents and the introgression lines (β -myrcene, 2-carene, α -phellandrene, α -terpinene, *p*-cymene, β -phellandrene, and γ -terpinene; Table II). Emission of α -phellandrene, α -terpinene, and *p*-cymene was significantly higher ($P < 0.001$, $P < 0.01$, and $P < 0.01$, respectively) in the lines with a higher repellence level compared to the Moneyberg plants and lines with low repellence scores (Table II).

In addition, *S. lycopersicum* ‘Moneymaker’ and three wild tomatoes (LA2560, PI127826, and GI1560) were analyzed for the production of green leaf volatiles (GLVs) by proton transfer reaction-mass spectrometry (Steeghs et al., 2006). Significant amounts of GLVs could only be detected after wounding the tomato plants (data not shown), ruling out the involvement of GLVs in *B. tabaci* host location.

Selected Semiochemicals and *B. tabaci* Behavior

To confirm the correlation between the seven candidate semiochemicals and *B. tabaci* behavior, host preference was assayed in bioassays with pure components. The selected pure volatile chemical(s) were administered on paper cards to Moneymaker plants (as described above). In the absence of added volatiles, the percentage of recaptured *B. tabaci* on each of the four tomato Moneymaker plants did not deviate significantly from the expected 25% (data not shown). However, when 10 μ g *p*-cymene was spotted on a filter paper and added to one of the plants, this plant became significantly ($P < 0.001$; Table III) less attractive to the whiteflies compared to the control plants. The percentage of whiteflies visiting the plant with *p*-cymene decreased with 44% on average, whereas the control plants harbored increased numbers of *B. tabaci*, as expected (Fig. 3A). Addition of 10 μ g β -myrcene on the other hand did not influence whitefly behavior (Fig. 3C). Application of 10 μ g γ -terpinene to the Moneymaker plants appeared to reduce attractiveness, but the difference was not significant (Fig. 3B; Table III). To assess whether a blend of these three components would yield a greater effect than *p*-cymene alone, a mixture of *p*-cymene: γ -terpinene: β -myrcene in the same ratio as found in accession LA2560 (1:12:3 [v/v]) was tested. This resulted in reduced attractiveness for whiteflies with 45% on average (Fig. 3D). The putative repellent semiochemicals α -phellandrene and α -terpinene, both monoterpenes that were identified with the introgression line

Table II. Selection of introgression lines and their parents *S. lycopersicum* 'Moneyberg' and *S. pennellii* accession LA716 ranked based on relative preference of *B. tabaci*

1 = Lowest preference; 5 = highest preference. Quantities of monoterpenes ($\mu\text{g } 24 \text{ h}^{-1} 10 \text{ g}^{-1}$ fresh weight) emitted. Values indicate means ($\pm \text{SE}$). *P* values indicate significant differences in monoterpene levels between lines with higher repellency ranking versus Moneyberg (MB) and line 1074. ns, Nonsignificant.

Line	Ranking	β -Myrcene	2-Carene	α -Phellandrene	α -Terpinene	<i>p</i> -Cymene	β -Phellandrene	γ -Terpinene
LA716	1	2.15 \pm 2.15	1.86 \pm 1.86	4.78 \pm 1.38	0.97 \pm 0.97	1.63 \pm 0.77	0.08 \pm 0.08	2.38 \pm 2.38
173	2	9.18 \pm 1.19	2.22 \pm 0.71	0.60 \pm 0.24	0.35 \pm 0.14	0.17 \pm 0.06	17.01 \pm 7.64	0.01 \pm 0.01
231	2	18.54 \pm 2.78	4.08 \pm 1.17	0.99 \pm 0.23	0.58 \pm 0.11	0.33 \pm 0.15	37.55 \pm 10.73	0.43 \pm 0.26
576	2	16.22 \pm 3.80	2.37 \pm 1.73	0.50 \pm 0.21	0.40 \pm 0.33	0.11 \pm 0.05	23.81 \pm 13.59	0.08 \pm 0.08
1126	2	9.03 \pm 1.44	0.72 \pm 0.13	0.24 \pm 0.10	0.03 \pm 0.03	0.05 \pm 0.01	4.96 \pm 0.85	0.0
1201	2	22.48 \pm 11.91	4.85 \pm 3.20	1.04 \pm 0.59	0.99 \pm 0.72	0.16 \pm 0.10	14.42 \pm 7.06	0.17 \pm 0.17
373	3	24.38 \pm 15.15	1.29 \pm 0.95	0.83 \pm 0.72	0.18 \pm 0.18	0.15 \pm 0.12	6.49 \pm 3.80	0.07 \pm 0.07
981	3	13.27 \pm 3.25	1.72 \pm 1.01	0.42 \pm 0.18	0.06 \pm 0.04	0.16 \pm 0.10	13.64 \pm 9.37	0.0
1007	3	20.96 \pm 8.86	3.98 \pm 1.98	0.93 \pm 0.45	0.80 \pm 0.46	0.19 \pm 0.11	40.95 \pm 21.57	0.11 \pm 0.11
1036	3	12.79 \pm 2.65	2.78 \pm 1.38	0.32 \pm 0.16	0.17 \pm 0.17	0.17 \pm 0.07	24.58 \pm 11.53	0.04 \pm 0.04
1073	4	19.09 \pm 3.45	1.45 \pm 0.30	0.69 \pm 0.33	0.27 \pm 0.02	0.14 \pm 0.03	10.46 \pm 1.61	0.04 \pm 0.04
1074	5	14.42 \pm 4.07	0.86 \pm 0.53	0.11 \pm 0.02	0.0	0.01 \pm 0.00	7.12 \pm 3.49	0.0
MB	5	5.43 \pm 0.48	0.35 \pm 0.06	0.0	0.0	0.02 \pm 0.00	2.60 \pm 0.33	0.0
<i>P</i> value		0.195 (ns)	0.140 (ns)	0.001	0.008	0.009	0.199 (ns)	0.357 (ns)

analyses, significantly decreased tomato attractiveness for whiteflies (Fig. 3, E and F; Table II). Finally, both sesquiterpenes that were tested, the *S. habrochaites*-derived zingiberene as well as its hydrogenation product curcumene, clearly reduced attractiveness (Fig. 3, G and H).

EAG

To investigate the responsiveness of *B. tabaci* to the selected semiochemicals, EAG was employed on whitefly antennae by puffing a 10^{-3} dilution of pure compounds over an isolated antenna and recording its physiological response. *B. tabaci* antennae responded to all compounds tested with the exception of β -myrcene (Table III). Comparably high responses of antennae to 10^{-4} dilutions of α -terpinene and curcumene (48 and 53 μV , respectively) indicate that *B. tabaci* antennae are able to detect these compounds even in low concentrations.

DISCUSSION

In this article, two functional metabolomic approaches led to the identification of several volatile mono- and sesquiterpenes from tomato that influence the whiteflies' choice for this host. The addition of some of these terpenes to the headspace of an otherwise attractive, cultivated tomato plant significantly reduced the number of whitefly visitations or, in other words, made the plant less preferred. Based on these observations, i.e. host selection based on odor cues instead of visual cues, together with a specific antennal response to certain terpenes, we propose a significant role for plants volatiles in the choice behavior of whiteflies.

Whiteflies Use Volatile Cues When Choosing a Host

The AFLP result presented in Supplemental Figure S1 confirms the previously reported genetic variation between whitefly populations (Frohlich et al., 1999). The invasive biotype B was even considered sufficiently different to be described as a new species (*Bemisia argentifolii* Bellows and Perring; Perring et al., 1993; Bellows et al., 1994), despite the morphological similarity to the indigenous biotype A. These distinct biotypes exhibit differential potential for virus transmission (Perring et al., 1993; Bedford et al., 1994). Interestingly, the B and Q biotype strains we tested preferred the same host in the choice assays (Fig. 1A), indicating some conservation of volatile perception and subsequent behavior.

Choice assays with tomato plants under a mesh cover indicated that whiteflies still make the same choice when only olfactory signals are offered (Fig. 1B). However, fewer whiteflies made a choice when

Table III. Bioactivity of seven candidate semiochemicals from wild tomato accessions in choice bioassays ($n = 8$) and electroantennographic responses (μV plus scatter, $n \geq 5$) of *B. tabaci* antennae (10^{-3} dilution in paraffin oil)

Source	Terpenoid	Repellent Effect	Antennal Response in μV (Range)
<i>S. pennellii</i>	<i>p</i> -Cymene	$P < 0.001$	24 (0–65)
	β -Myrcene	$P = 0.479$	nr
	γ -Terpinene	$P = 0.102$	19 (5–36)
	α -Terpinene	$P = 0.014$	41 (3–126)
<i>S. habrochaites</i>	α -Phellandrene	$P = 0.030$	22 (0–78)
	Zingiberene	$P < 0.001$	23 (0–53)
	Curcumene	$P < 0.001$	50 (8–148)

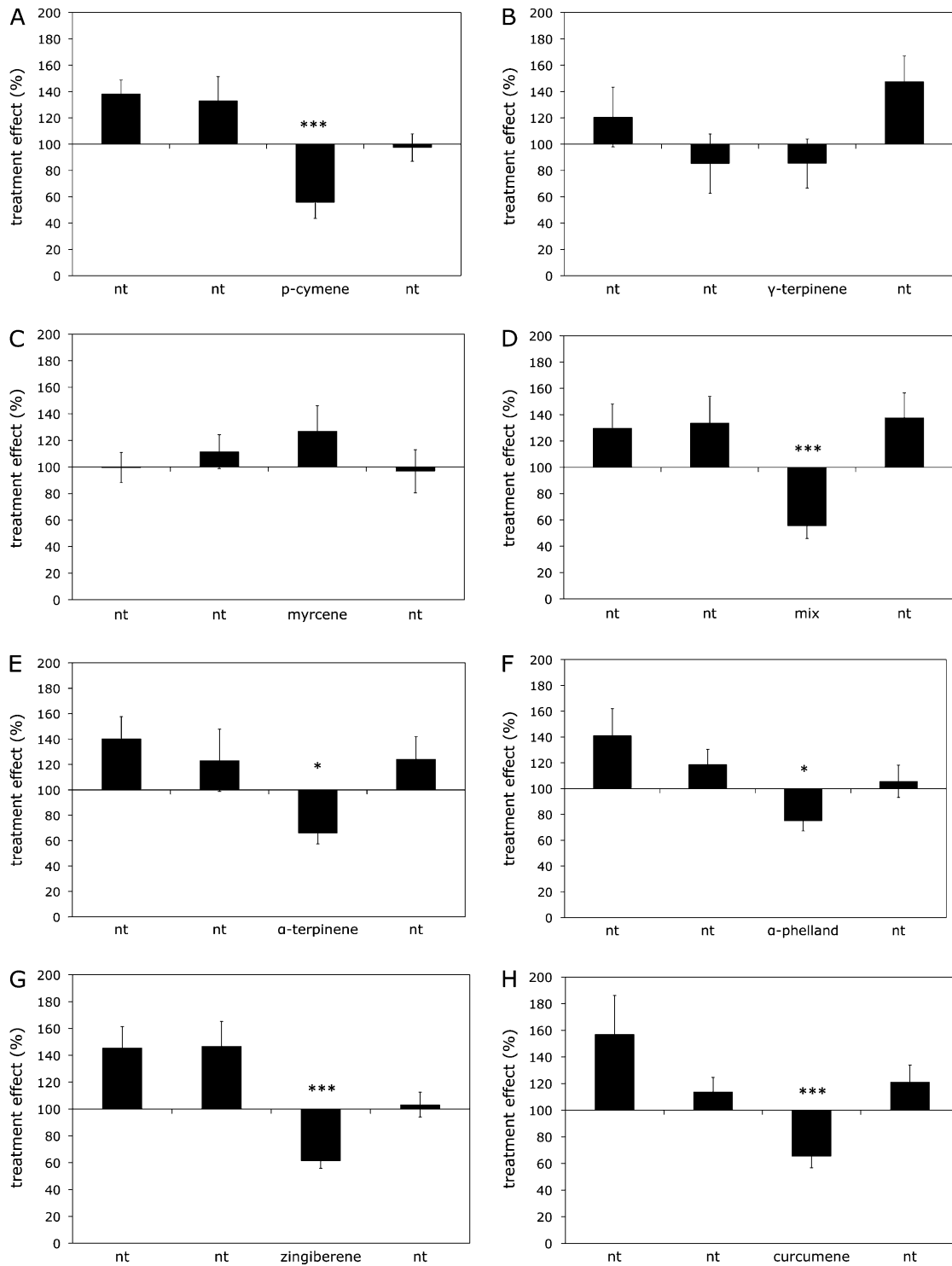


Figure 3. Specific tomato volatile compounds can repel whiteflies. Effect of one volatile compound (or mix) added to one plant in a setup of four *S. lycopersicum* ‘MoneyMaker’ plants (expressed as percentage of control setup, $n = 200$) on *B. tabaci* biotype Q choice behavior. Addition of 10 μg : *p*-cymene (A), γ -terpinene (B), β -myrcene (C), a mix of *p*-cymene, γ -terpinene, and β -myrcene (D), α -terpinene (E), α -phellandrene (F), zingiberene (G), and curcumene (H). Bars represent averaged values of eight experiments ($\pm\text{SE}$) nt, Plants in setup to which no volatiles were added. Level of significance: *, $P < 0.05$; ***, $P < 0.001$.

the plants were covered, indicating that locating hosts effectively requires multimodal sensory signals, including visual cues.

Tomato Volatile Semiochemicals Can Influence Whitefly Choice Behavior

From previous studies it was known that the wild tomatoes *S. pennellii* and *S. habrochaites* (former *Lycopersicon hirsutum*) are less preferred by *B. tabaci* than cultivated tomato varieties, which was attributed to the production of volatiles by their glandular trichomes (Simmons and Gurr, 2005). Targeted approaches showed that undecanone, a fatty acid derivative (Fridman et al., 2005), and the sesquiterpene zingiberene (Antonious and Kochhar, 2003), both produced in the trichomes, were toxic to whiteflies (Freitas et al., 2002; Antonious et al., 2005). We decided to investigate the influence of the tomato headspace volatiles on the initial choice behavior of the whiteflies using short-choice assays of 10 min. In this way, the repellent effects of the headspace volatiles could be separated from gustatory cues after host contact. *B. tabaci* exhibited a clear preference for cultivated tomato varieties over wild tomatoes (Table I). It is known that airborne semiochemicals can promote or deter interactions between plants and herbivorous insects (reviewed in Bruce et al., 2005a). We showed that the addition of the mixture of volatile compounds present in the headspace of the two least preferred tomato accessions (LA2560 and PI127826) to an otherwise attractive cultivated tomato plant significantly reduced whitefly visitations (Fig. 2), showing that manipulation of olfactory cues can in fact alter the attractiveness of a host plant for *B. tabaci*.

Our untargeted approach of correlating the headspace profiles of 21 different tomato lines with their relative attractiveness revealed only terpenes as putative semiochemicals in the tomato-whitefly interaction. Among these were zingiberene and curcumenone (Table I), which are apparently not only toxic (Weston et al., 1989) but also have a role as volatile cue. The major constituents of *S. habrochaites* f. *glabratum* trichomes are methylketones (Antonious et al., 2005), well known for their role in plant-insect interactions. However, with our approach, no correlation between the presence of methylketones and whitefly behavior was found.

Another important group of volatile components, the green leaf volatiles, were excluded from a role as semiochemicals in the initial whitefly-tomato interaction, as we could not detect these compounds unless tomatoes were mechanically damaged (data not shown). Terpenes are known to be constituents of essential plant oils with ecological roles in plant-insect relations, including a role as insect repellents (Bruce et al., 2005b; John et al., 2006; Van Tol et al., 2007). Previously, other hemiptera have been shown to be repelled by plant produced volatiles (Gibson and Pickett, 1983), and *Myzus persicae* behavioral response could be modified by altering plant terpene produc-

tion (Beale et al., 2006). Because of the interesting repellence levels in all of the three accessions of *S. pennellii* tested, a *S. pennellii* × *S. lycopersicum* introgression library was screened in an additional approach to identify active semiochemical compounds (Table II; Supplemental Fig. S3). This experiment confirmed the monoterpene *p*-cymene as putatively repellent and revealed two additional candidates, i.e. α -terpinene and α -phellandrene.

Specific Terpenes Can Make a Host Less Attractive

The olfactory receptor neurons in an insect's antennae will not only detect the ratio of compounds for recognition of a host (Webster et al., 2008) but can also identify nonhost compounds acting as deterrents or alarm signals that might mask the attractiveness of the host plant (Nottingham et al., 1991). True repellence relates to a behavioral effect caused by the perception of a component at the peripheral sensory nervous system, which refrains the insect from probing and causes avoidance behavior away from the source of repellence (Pickett et al., 2008). Here, we scored repellence as a change in host choice when compounds were added to the headspace.

By correlation of headspace analyses and behavioral assays, two sesquiterpenes, zingiberene and curcumenone, were identified as putatively involved in the whitefly-tomato interaction (Table I). This was corroborated in the reconstitution experiments (Fig. 3, G and H). In addition, EAG analyses confirmed both compounds to be recognized by whitefly antennae (Table III). Zingiberene readily transforms to curcumenone in the presence of air (Chen and Ho, 1988). Moreover, the sensitivity of the whitefly antenna appears to be higher for curcumenone (Table III), i.e. even though a blend of the two terpenes can be expected, we speculate that curcumenone is detected at further distance from the odor source and therefore is most likely more responsible for the deterring effect. The toxicity of zingiberene and curcumenone to insects has been shown previously in experiments with essential oils and extracts (Carter et al., 1989; Weston et al., 1989; Eigenbrode et al., 1994; Freitas et al., 2002; de Azevedo et al., 2003). Freitas et al. (2002) also showed that there is a positive and heritable relation between tomato foliar zingiberene levels and levels of *B. argentifolii* infestation. However, this correlation was scored for nymph survival in a no-choice experiment and relates to the toxic properties of zingiberene rather than to its repellent effects. Foliar application of ginger oil, containing zingiberene as the major component, appeared to be effective in protecting tomato against whitefly (Zhang et al., 2004). In their experimental setup, ginger oil appeared to repel whiteflies only at distance <1 mm from the odor source. Here, we show that treatment of a visually unaltered host with zingiberene/curcumenone purified from wild tomato results in significant avoidance behavior, indicating that specific

olfactory cues are indeed an important step in host location from distance.

Of the monoterpenes associated with repellence in correlation analyses, *p*-cymene, α -terpinene, and α -phellandrene were positively identified as repellent compounds in bioassays (Fig. 3, A, E, and F, respectively). A mixture of *p*-cymene, γ -terpinene, and β -myrcene in ratios found in the most repellent *S. pennellii* accession LA2560 did not provide a stronger repellent effect than *p*-cymene alone (Fig. 3D), indicating that *p*-cymene is the active compound in this plant's volatile profile. There is ample literature on the role of monoterpenes as toxins or repellents. Previously, *p*-cymene has been reported to be a toxic agent for the western flower thrips (*Frankliniella occidentalis*; Janmaat et al., 2002) and a repellent against mosquitoes (Park et al., 2003). Terpinenes and phellandrenes can be converted into *p*-cymene both enzymatically and through hydrogenation (LaFever and Croteau, 1993). Therefore, we hypothesize that *p*-cymene is the active end product in whitefly repellence. EAG analyses identified α -terpinene as the monoterpene that elicited the highest response in whitefly antennae (Table III), meaning the insect will sense this compound even when present at low concentrations. Remarkably, β -myrcene did not evoke a response in the antennae, indicating that not all volatiles in the tomato headspace are recognized by *B. tabaci*.

Apparently, *B. tabaci* antennae are able to identify specific terpenes in their host headspace to which they zoom in. Odor cues might indicate toxic or other life-threatening environments. Whiteflies were found to be able to select their host plant based not on host quality, but possibly associated to predator related cues (Nomikou et al., 2003). So far, most research has focused on the action of terpenes as toxins or feeding deterrents. In the case of virus-transmitting insects, altering choice behavior prior to the insect's landing and feeding is crucial to avoid the devastating effects of infection. Although new approaches with transgenic plants inhibiting viral transmission by whiteflies are also promising (Akad et al., 2007), by making use of natural plant terpenoid production, plant-insect interactions could be manipulated in such a way that it alters insect behavior and dramatically decreases the plant's attractiveness (Degenhardt et al., 2003; Kappers et al., 2005; Schnee et al., 2006), whereas increase in other terpenes might improve resistance through the attraction of natural enemies, as described recently by Köllner et al. (2008). Breeding efforts to increase the emission of specific volatile terpenes might decrease whitefly infestation.

MATERIALS AND METHODS

Tomato and *Bemisia tabaci* Material and Rearing Conditions

Tomato (*Solanum* spp.) accessions spanning a variety of different types and cultivars were either provided by Dutch seed companies (via Keygene) or

ordered via the C.M. Rick Tomato Genetics Resource Center (Davis, CA) and grown in a greenhouse compartment (22–25°C, 16/8 h photoperiod at 500 $\mu\text{E m}^{-2} \text{s}^{-1}$). The collection consisted of five cultivated tomatoes (*Solanum lycopersicum*; cv Moneymaker, cv Mogeor, cv Motelle, cv Monalbo, and cv Pitenza) and 16 wild tomatoes, among which were *Solanum pennellii* (accessions LA2560, LA716, and LA1340), *Solanum habrochaites* former f. *typicum* (accessions LA1777, LA1553, PI127826, and PI127827) and former f. *glabratum* (accessions PI126449, PI134417, PI134418, PI251304, IVT701631, LA407, GI1560, and LA1840), and *Solanum peruvianum* (accession LA1708). To measure *B. tabaci* response and measure headspace volatiles in the same genetic background, plants were propagated by cuttings. For a second approach, a *S. pennellii* LA716 \times *S. lycopersicum* 'Moneyberg' introgression population (Keygene) was screened for *B. tabaci* response. The introgression library (72 lines) was built from three backcrosses to the recurring parent and from here on, individual plants were selfed until homozygosity of the donor fragments was achieved. The headspace volatiles were determined by gas chromatography-mass spectrometry analyses as described below for both parents as well as for a subset of 11 introgression lines.

A population of *B. tabaci* used in the bioassays was collected at a production greenhouse in Santa María del Águila (Almería province, Spain) in October, 2005. The population was reared in a climatized chamber (Snijders Tilburg; temperature 28°C, 16 h light, 150 $\mu\text{E m}^{-2} \text{s}^{-1}$, relative humidity 75%) on cucumber (*Cucumis sativus*). A second population originated from the floriculture research station in Aalsmeer, The Netherlands (Labculture Netherlands).

Genetic Diversity Assessment of *B. tabaci*

Individuals of 33 whitefly populations from crop production areas all over the world were collected in 70% ethanol. DNA was isolated with a DNeasy kit for insect DNA purification (Qiagen Benelux) according to the manufacturer's instructions. The DNA was digested using *EcoRI* and *MseI* restriction enzymes resulting in *EcoRI/MseI* templates. Primer combinations E39/M64, E82/M59, E35/M66, E35/M70, E92/M48, and E92/M61 were selected for the AFLP screen (www.keygene.com/keygene/pdf/PRIMERCO.pdf). Fingerprinting using these primer combinations resulted in a data set of 412 markers that were dominantly scored. The marker score was used to carry out the diversity assessment analyses to categorize the individuals on basis of genetic similarity. NTSYSpc software (Exeter Software; <http://www.exetersoftware.com>) was used to produce three similarity matrices consisting of similarity indices for all combinations of samples. The genetic similarity matrix was calculated according to the Jaccard coefficient ($J = a/n - d$). To visualize the relationship between samples, dendrograms were generated using sequential agglomerative hierarchical nested cluster analysis based on the unweighted pair-group method of arithmetic averages. To evaluate the extent of representation of the similarity matrix by the dendrogram, a cophenetic value matrix was calculated. The cophenetic correlations for all dendrograms are between 0.96 and 0.98, which implies that the dendrograms are highly representative of the similarities between the populations.

The biotypes of the two live *B. tabaci* populations used (Almería B and Labculture Netherlands) were confirmed using a cleaved-amplified polymorphic sequence marker for cytochrome oxidase I (mtCOI) as described by Khasdan et al. (2005). PCR amplification was performed using primers C1-J-2159 and L2-N-3014 (Frohlich et al., 1999), which was followed by digestion with *VspI*, generating a clear polymorphism between biotype B (Labculture Netherlands) and biotype Q (Almería population).

Tomato Headspace Sampling

Volatiles were collected by placing 3-week-old wild tomato plants in a climatized room in large desiccators for 24 h, including a 16-h day period. Desiccators were ventilated with carbon-filtered pressure air at 400 mL min^{-1} . Volatiles were captured on a sampling tube containing 300 mg Tenax resin according to Kant et al. (2004). Next, volatiles were eluted off the Tenax with 1 mL pentane:diethylether (4:1), including benzoic acid as an internal standard. Identification was achieved by injecting 1 μL of the eluent into an optic injection port (ATAS GL International) at 50°C, which was heated to 275°C at a rate of 4°C s^{-1} . The split flow was 0 mL for 2 min and then 25 mL min^{-1} . Compounds were separated on a capillary DB-5 column (10 \times 180 μm , film thickness 0.18 μm ; Hewlett-Packard) at 40°C for 3 min and then to 250°C at 30°C min^{-1} with helium as a carrier gas. The column flow was 3 mL min^{-1} for 2 min and 1.5 mL min^{-1} thereafter. Mass spectra of eluting compounds were

generated at 70 eV (ion source at 200°C) and collected on a time-of-flight mass spectrometer (Leco Pegasus III) with a 90-s acquisition delay at 1597 eV, at an acquisition rate of 20 spectra s^{-1} . Sample identification and quantification was based on synthetic external standards of known concentration (Fluka). Each tomato accession was measured six times.

Free-Choice Bioassays with *B. tabaci* and Tomato

Free-choice experiments with *B. tabaci* were carried out in a greenhouse compartment (28°C, relative humidity 65%). Light was supplied by high-pressure sodium lamps (Hortilux Schröder SON-T PIA GP 600W) with a photosynthetic irradiance of 250 $W m^{-2}$. The preference behavior of *B. tabaci* biotype Q (Almería population) and biotype B (Labculture Netherlands) was compared in bioassays with different wild tomatoes (LA1777, LA2560, GI1560, and *S. lycopersicum* 'MoneyMaker'). Three-week-old plants were placed inside a plastic-covered wooden tray (170 × 100 × 20 cm) filled with soil, at equal distance from each other. Two or three hundred adult whiteflies were captured, placed at 4°C for 5 min, and then released in the middle of the setup. Ten and 20 min after release, settled whiteflies were recaptured, and the numbers of whiteflies on each plant were recorded. For each experiment, new, inexperienced whiteflies were used. Since no differential behavior between *B. tabaci* B and Q was found, all further bioassays were done with the Almería (Q) population. To test whether choice behavior would change in the absence of visual cues, a set of bioassays as described above was repeated with plants individually placed under dark-gray mesh cover (mesh size; 1 mm) (Supplemental Fig. S2). Four very similar 3-week-old cultivated *S. lycopersicum* plants were used for this experiment: cv Motelle (contains *Mi1.2*), cv Monalbo, cv Mogeor (contains *Mi1.2*), and cv Pitenza. First, the distribution of *B. tabaci* in a setup without mesh covers was ascertained, after which the experiment was repeated with all four plants covered. The pore size of the mesh was big enough for whiteflies to go through, but they were also counted as having made a choice (10 min after release) if they were on the mesh of a particular tomato. The experiment was repeated three times. In the presence of visual cues, on average 75% of the whiteflies released ($n = 200$) were recovered within 20 min, compared to 46% in the absence of visual cues.

To rank the preference of whiteflies for each plant (16 wild and five cultivated), stepwise sequential testing was applied. Six randomly chosen plants were placed inside a plastic-covered wooden tray as described above. Three hundred whiteflies were released in the middle and recaptured from plants after 10 and 20 min, and the distribution was established. Each bioassay was repeated three times with randomized positions of the plants. After each bioassay, the two most attractive plants were replaced by two randomly chosen new plants for a next set of bioassays. This procedure was repeated until all plants were tested. Finally, all plants were retested in a setup with plants with similar repellence scores (again in sets of six) to yield the final ranking.

For bioassays with headspace (total headspace or single compounds), four potted tomato plants (*S. lycopersicum* 'MoneyMaker') were placed in a square setup at a distance of 50 cm from each other. Two hundred whiteflies were released and recorded as described above. To test the effect of selected headspace components on repellence, 10 μg of synthetic standards (Fluka) were applied to 10 filter paper discs (Whatman; 25-mm diameter). The treated discs were attached to one of the four plants with metal wire. The position of the treated tomato was randomized. Five minutes after placing the volatiles on the plant, whiteflies were released. For each component, eight replications were carried out. Treatments were administered in a setup in which compounds were always added to the same plant in comparison to three plants with empty carriers. Prior to each assay with volatiles, the experiment was done with the four plants in an untreated setup, in the same position to allow comparison in the same background. For each volatile compound tested, a new set of plants was used.

EAG

A dose-response series of EAG responses of *B. tabaci* were measured by manual injection of synthetic standards (Fluka) into a humidified air stream passing over the antenna of *B. tabaci* mounted onto an insect antenna holder, which was housed in a polytetrafluoroethylene cell (flow rate of 250 mL/min). The antenna holder was milled from a perspex disc and adapted to the size of the antennae (Thakeow et al., 2008). Within this holder, the ends of the excised antennae contacted an electrolyte solution that provided electrical contact to a pair of Ag/AgCl electrodes. The EAG potentials were amplified by a factor of

100 using a high impedance amplifier (input impedance 100 M Ω ; U.T. Koch, Kaiserslautern, Germany) with a built-in low-pass filter set to a cutoff frequency of 1 Hz to suppress the ubiquitous electrical supply frequency of 50 Hz. The amplified and filtered signal was digitized using a 35900E A/D converter (Agilent) and recorded by the GC ChemStation software (Agilent; Weißbecker et al., 2004). Odor standards were produced from a dilution series of the respective synthetic standards in paraffin oil (Uvasol-quality; Merck/VWR). Small pieces of filter paper (2 cm; Schleicher & Schuell) were soaked with 100 μL of the standard dilution or paraffin oil only (control). The filter paper was put into a 10-mL glass syringe (Poulten & Graf). Inside the air volume of the syringe, the odorant accumulated at a concentration proportional to the concentration of the substance in the solution and its vapor pressure according to Henry's law. A reproducible stimulus could be supplied by puffing 5 mL of air over the antenna (Schütz et al., 1999). Oil dilutions of the synthetic standards to 10^{-3} and 10^{-4} were measured at least five times with at least five different insects each in a randomized sequence.

Statistical Analyses

Absence, presence, and concentration of terpenes in different headspaces were correlated to repellence rank by multiple-linear regression analyses, and statistical significance was tested by multivariate ANOVA and nonparametric analyses. In the past few years, random forests have become a popular and widely used tool for nonparametric regression in many scientific areas. Random forest was used to validate the ranking order and to identify relevant volatiles influencing the rank variable. The random forest procedure does not require any distributional assumption because it is not model based but algorithm based (Faraway, 2006). Effect of headspace (components) to repellence was tested by one- and two-way ANOVA followed by Tukey's test for comparison of individual means. When necessary, values were log transformed prior to analysis.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Dendrogram based on AFLP polymorphisms generated using the Jaccard algorithm categorizing the different *B. tabaci* populations by their genetic correlation.

Supplemental Figure S2. Mesh cover (left) placed over a tomato plant, as used in the experimental setup (see Fig. 1B), concealing its visual cues for whiteflies.

Supplemental Figure S3. Relative *B. tabaci* (biotype Q) attraction (% $n = 200$) to 72 introgression lines (*S. lycopersicum* 'Moneyberg' × *S. pennellii* LA716; dark bars) compared to both parents (light bars; Moneyberg [MB] and LA716).

Supplemental Table S1. List of compounds detected in wild tomato accessions (*S. pennellii*, *S. habrochaites*, and *S. peruvianum*) and cultivated tomato lines (*S. lycopersicum*).

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LITERATURE CITED

- Akad F, Eybishtz A, Edelbaum D, Gorovits R, Dar-Issa O, Iraki N, Czosnek H (2007) Making a friend from a foe: expressing a GroEL gene from the whitefly *Bemisia tabaci* in the phloem of tomato plants confers resistance to tomato yellow leaf curl virus. *Arch Virol* 152: 1323–1339
- Antonious GF, Kochhar TS (2003) Zingiberene and curcumen in wild tomato. *J Environ Sci Health B* 38: 489–500
- Antonious GF, Kochhar TS, Simmons AM (2005) Natural products:

- seasonal variation in trichome counts and contents in *Lycopersicon hirsutum* f. *glabratum*. *J Environ Sci Health B* **40**: 619–631
- Beale MH, Birkett MA, Bruce TJA, Chamberlain K, Field LM, Huttly AK, Martin JL, Parker R, Philips AL, Pickett JA, et al (2006) Aphid alarm pheromone produced by transgenic plants affects aphid and parasitoid behavior. *Proc Natl Acad Sci USA* **103**: 10509–10513
- Bedford ID, Briddon RW, Brown JK, Rosell RC, Markham PG (1994) Geminivirus transmission and biological characterisation of *Bemisia tabaci* (Gennadius) biotypes from different geographical regions. *Ann Appl Biol* **125**: 311–325
- Belliure B, Janssen A, Sabelis MW (2008) Herbivore benefits from vectoring plant virus through reduction of period of vulnerability to predation. *Oecologia* **156**: 797–806
- Bellows TS, Perring TM, Gill RJ, Headrick DH (1994) Description of a species of *Bemisia* (Homoptera: Aleyrodidae). *Ann Entomol Soc Am* **87**: 195–206
- Birkett MA, Agelopoulos N, Jensen KMV, Jespersen JB, Pickett JA, Prijs HJ, Thomas G, Trapman JJ, Wadhams LJ, Woodcock CM (2004) The role of volatile semiochemicals in mediating host location and selection by nuisance and disease-transmitting cattle flies. *Med Vet Entomol* **18**: 313–322
- Bruce TJA, Birkett MA, Blande J, Hooper AM, Martin JL, Khambay B, Prosser I, Smart LE, Wadhams LJ (2005b) Response of economically important aphids to components of *Hemizygia petiolata* essential oil. *Pest Manag Sci* **61**: 1115–1121
- Bruce TJA, Wadhams LJ, Woodcock CM (2005a) Insect host location: a volatile situation. *Trends Plant Sci* **10**: 269–274
- Carter CD, Sacalis JN, Gianfagna TJ (1989) Zingiberene and resistance to Colorado potato beetle in *Lycopersicon hirsutum* f. *hirsutum*. *J Agric Food Chem* **37**: 206–210
- Chen CC, Ho CT (1988) Gas-chromatographic analysis of volatile components of ginger oil (Zingiber-officinale Roscoe) extracted with liquid carbon-dioxide. *J Agric Food Chem* **36**: 322–328
- de Azevedo SM, Faria MV, Maluf WR, Barneche de Oliveira AC, De Freitas JA (2003) Zingiberene-mediated resistance to the South American tomato pinworm derived from *Lycopersicon hirsutum* var. *hirsutum*. *Euphytica* **134**: 347–351
- Degenhardt J, Gershenson J, Baldwin IT, Kessler A (2003) Attracting friends to feast on foes: engineering terpene emission to make crop plants more attractive to herbivore enemies. *Curr Opin Biotechnol* **14**: 169–176
- De Moraes CM, Lewis WJ, Paré PW, Alborn HT, Tumlinson JH (1998) Herbivore-infested plants selectively attract parasitoids. *Nature* **393**: 570–573
- Drake JW, Holland JJ (1999) Mutation rates among RNA viruses. *Proc Natl Acad Sci USA* **96**: 13910–13913
- Eigenbrode SD, Trumble JT, Millar JG, White KK (1994) Topical toxicity of tomato sesquiterpenes to the beet armyworm and the role of these compounds in resistance derived from an accession of *Lycopersicon hirsutum* f. *typicum*. *J Agric Food Chem* **42**: 807–810
- Faraway JJ (2006) Extending the Linear Model with R: Generalized Linear, Mixed Effects and Nonparametric Regression Models. *Texts in Statistical Science*. Chapman & Hall/CRC, Boca Raton, FL, pp 253–261
- Freitas JA, Maluf WR, Graças Cardoso M, Gomes LAA, Bearzotti E (2002) Inheritance of foliar zingiberene contents and their relationship to trichome densities and whitefly resistance in tomatoes. *Euphytica* **127**: 275–287
- Fridman E, Wang J, Iijima Y, Froehlich JE, Gang RR, Ohlrogge J, Pichersky E (2005) Metabolic, genomic and biochemical analyses of glandular trichomes from the wild tomato species *Lycopersicon hirsutum*. Identify a key enzyme in the biosynthesis of methylketones. *Plant Cell* **17**: 1252–1267
- Frohlich DR, Torres-Jerez I, Bedford ID, Markham PG, Brown JK (1999) A phylogeographical analysis of the *Bemisia tabaci* species complex based on mitochondrial DNA markers. *Mol Ecol* **8**: 1683–1691
- García-Andrés S, Monci F, Navas-Castillo J, Moriones E (2006) Begomovirus genetic diversity in the native plant reservoir *Solanum nigrum*: evidence for the presence of a new virus species of recombinant nature. *Virology* **350**: 433–442
- Ghanim M, Morin S, Zeidan M, Czosnek H (1998) Evidence for transovarial transmission of tomato yellow leaf curl virus by its vector, the whitefly *Bemisia tabaci*. *Virology* **240**: 295–303
- Gibson RW, Pickett JA (1983) Wild potato repels aphids by release of aphid alarm pheromone. *Nature* **302**: 608–609
- Horowitz AR, Kotsedalov S, Khasdan V, Ishaaya I (2005) Biotypes B and Q of *Bemisia tabaci* and their relevance to neonicotinoid and pyriproxyfen resistance. *Arch Insect Biochem Physiol* **58**: 216–225
- Inbar M, Gerling D (2008) Plant-mediated interactions between whiteflies, herbivores, and natural enemies. *Annu Rev Entomol* **53**: 431–448
- Janmaat AF, De Kogel WJ, Woltering EJ (2002) Enhanced fumigant toxicity of *p*-cymene against *Frankliniella occidentalis* by simultaneous application of elevated levels of carbon dioxide. *Pest Manag Sci* **58**: 167–173
- Jiu M, Zhou XP, Tong L, Xu J, Yang X, Wan FH, Liu SS (2007) Vector-virus mutualism accelerates population increase of an invasive whitefly. *PLoS One* **2**: 1–8
- Johne BA, Weissbecker B, Schütz S (2006) Volatile emissions from *Aesculus hippocastanum* induced by mining of larval stages of *Cameraria ohridella* influence oviposition by conspecific females. *J Chem Ecol* **32**: 2303–2319
- Jones DR (2003) Plant viruses transmitted by whiteflies. *Eur J Plant Pathol* **109**: 195–219
- Kant MR, Ament K, Sabelis MW, Haring MA, Schuurink RC (2004) Differential timing of spider mite-induced direct and indirect defenses in tomato plants. *Plant Physiol* **135**: 483–495
- Kappers IE, Aharoni A, Van Herpen TW, Luckerhoff LL, Dicke M, Bouwmeester HJ (2005) Genetic engineering of terpenoid metabolism attracts bodyguards to Arabidopsis. *Science* **309**: 2070–2072
- Kennedy GG (2003) Tomato, pests, parasitoids, and predators: tritrophic interactions involving the genus *Lycopersicon*. *Annu Rev Entomol* **48**: 51–72
- Kessler A, Baldwin IT (2001) Defensive function of herbivore-induced plant volatile emissions in nature. *Science* **291**: 2141–2144
- Khasdan V, Levin I, Rosner A, Morin S, Kotsedalov S, Maslennik L, Horowitz AR (2005) DNA markers for identifying biotypes B and Q of *Bemisia tabaci* (Hemiptera: Aleyrodidae) and studying population dynamics. *Bull Entomol Res* **95**: 605–613
- Köllner TG, Schnee C, Li S, Svatos A, Schneider B, Gershenson J, Degenhardt J (2008) Protonation of a neutral (S)- β -bisabolene intermediate is involved in (S)- β -macrocyclic formation by the maize sesquiterpene synthases TPS6 and TPS11. *J Biol Chem* **283**: 20779–20788
- LaFever RE, Croteau R (1993) Hydride shifts in the biosynthesis of the p-menthane monoterpenes alpha-terpinene, gamma-terpinene, and beta-phellandrene. *Arch Biochem Biophys* **301**: 361–366
- Maruthi MN, Colvin J, Seal S (2001) Mating compatibility, life-history traits, and RAPD-PCR variation in *Bemisia tabaci* associated with the cassava mosaic disease pandemic in East Africa. *Entomol Exp Appl* **99**: 13–23
- Maruthi MN, Czosnek H, Vidavski F, Tarba SY, Milo J, Leviatov S, Venkatesh HM, Padmaja AS, Kulkarni RS, Muniyappa V (2003) Comparison of resistance to Tomato leaf curl virus (India) and Tomato yellow leaf curl virus (Israel) among *Lycopersicon* wild species, breeding lines and hybrids. *Eur J Plant Pathol* **109**: 1–11
- Medeiros RB, Resende RO, de Avila AC (2004) The plant virus tomato spotted wilt tospovirus activates the immune system of its main insect vector, *Frankliniella occidentalis*. *J Virol* **78**: 4976–4982
- Nombela G, Williamson VM, Muniz M (2003) The root-knot nematode resistance gene Mi-1.2 of tomato is responsible for resistance against the whitefly *Bemisia tabaci*. *Mol Plant Microbe Interact* **16**: 645–649
- Nomikou M, Janssen A, Sabelis MW (2003) Herbivore host plant selection: whitefly learns to avoid host plants that harbour predators of her offspring. *Oecologia* **136**: 484–488
- Nomikou M, Janssen A, Schraag R, Sabelis MW (2002) Phytoseiid predators suppress populations of *Bemisia tabaci* on cucumber plants with alternative food. *Exp Appl Acarol* **27**: 57–68
- Nottingham SF, Hardie J, Dawson GW, Hick AJ, Pickett JA, Wadhams LJ, Woodcock CM (1991) Behavioural and electrophysiological responses of aphids to host and nonhost plant volatiles. *J Chem Ecol* **17**: 1231–1242
- Park IK, Lee SG, Choi DH, Park JD, Ahn YJ (2003) Insecticidal activities of constituents identified in the essential oil from leaves of *Chamaecyparis obtusa* against *Callosobruchus chinensis* (L.) and *Sitophilus oryzae* (L.). *J Stored Prod Res* **39**: 375–384
- Perring TM, Cooper AD, Rodriguez RJ, Farr CA, Bellows TS (1993) Identification of a whitefly species by genomic and behavioral studies. *Science* **259**: 74–77
- Peterson CJ, Coats JR (2001) Insect repellents – past, present and future. *Pestic Outlook* **12**: 154–158

- Peterson CJ, Nemetz LT, Jones LM, Coats JR (2002) Behavioral activity of Catnip (*Lamiaceae*) essential oil components to the German Cockroach (*Blattodea: Blattellidae*). *J Econ Entomol* **95**: 377–380
- Pichersky E, Noel JP, Dudareva N (2006) Biosynthesis of plant volatiles: nature's diversity and ingenuity. *Science* **311**: 808–811
- Pickett JA, Birkett MA, Logan JG (2008) DEET repels ORNery mosquitoes. *Proc Natl Acad Sci USA* **105**: 13195–13196
- Polston JE, Anderson KP (1997) The emergence of whitefly-transmitted geminiviruses in tomato in the western hemisphere. *Plant Dis* **81**: 1358–1369
- Rosell RC, Torres-Jerez I, Brown JK (1999) Tracing the geminivirus-whitefly transmission pathway by polymerase chain reaction in whitefly extracts, saliva, hemolymph, and honeydew. *Phytopathology* **89**: 239–246
- Sacchetti JC, Poulter CD (1997) Creating isoprenoid diversity. *Science* **277**: 1788–1789
- Schnee C, Köllner TG, Held M, Turlings TC, Gershenzon J, Degenhardt J (2006) The products of a single maize sesquiterpene synthase form a volatile defense signal that attracts natural enemies of maize herbivores. *Proc Natl Acad Sci USA* **103**: 1129–1134
- Schütz S, Weißbecker B, Klein A, Hummel HE (1997) Host plant selection of the Colorado potato beetle as influenced by damage induced volatiles of the potato plant. *Naturwissenschaften* **84**: 212–217
- Schütz S, Weißbecker B, Koch UT, Hummel HE (1999) Detection of volatiles released by diseased potato tubers using a biosensor on the basis of intact insect antennae. *Biosens Bioelectron* **14**: 221–228
- Shiojiri K, Kishimoto K, Ozawa R, Kugimiya S, Urashimo S, Arimura G, Horiuchi J, Nishioka T, Matsui K, Takabayashi J (2006) Changing green leaf volatile biosynthesis in plants: an approach for improving plant resistance against both herbivores and pathogens. *Proc Natl Acad Sci USA* **103**: 16672–16676
- Simmons AM (1994) Oviposition on vegetables by *Bemisia tabaci* (Homoptera: Aleyrodidae): temporal and leaf surface factors. *Environ Entomol* **23**: 381–389
- Simmons AM, Gurr GM (2005) Trichomes of *Lycopersicon* species and their hybrids: effects on pests and natural enemies. *Agric For Entomol* **7**: 265–276
- Snyder JC, Guo Z, Thacker R, Goodman JP, Pyrek JST (1993) 2,3-Dihydrofarnesoic acid, a unique terpene from trichomes of *Lycopersicon hirsutum* repels spider mites. *J Chem Ecol* **19**: 2981–2997
- Steeghs MM, Moeskops BWM, Van Swam K, Cristescu SM, Scheepers PTJ, Harren FJM (2006) On-line monitoring of UV-induced lipid peroxidation products from human skin in vivo using proton-transfer reaction mass spectrometry. *Int J Mass Spectrom* **253**: 58–64
- Terry I, Walter GH, Moore C, Roemer R, Hull C (2007) Odor-mediated push-pull pollination in cycads. *Science* **318**: 70
- Thakeow P, Angeli S, Weißbecker B, Schütz S (2008) Antennal and behavioural responses of the fungivorous insect *Cis boleti* to volatiles of its host fungus. *Chem Senses* **33**: 379–387
- Van Lenteren JC, Noldus LPJJ (1990) Whitefly-plant relationships: behavioural and ecological aspects. In D Gerling, ed, *Whiteflies: Their Biology, Pest Status and Management*. Intercept Ltd., Andover, UK, pp 47–49
- Van Poecke RM, Dicke M (2002) Induced parasitoid attraction by *Arabidopsis thaliana*: involvement of the octadecanoid and the salicylic acid pathway. *J Exp Bot* **53**: 1793–1799
- Van Tol RW, Swarts HJ, Van der Linden A, Visser JH (2007) Repellence of the red bud borer *Resseliella oculiperda* from grafted apple trees by impregnation of rubber budding strips with essential oils. *Pest Manag Sci* **63**: 483–490
- Visser JH (1988) Host-plant finding by insects: orientation, sensory input and search patterns. *J Insect Physiol* **34**: 259–268
- Wan FH, Xie M, Cui XH (2008) Effects of heat shock on survival and fecundity of two whitefly species, *Trialeurodes vaporariorum* (Westwood) and *Bemisia tabaci* (Gennadius) B-biotype. In PA Stansly, CL McKenzie, eds, *Fourth International Bemisia Workshop International Whitefly Genomics Workshop*. *J Insect Sci* **8**: 4
- Webster B, Bruce T, Dufour S, Birkemeyer C, Birkett M, Hardie J, Pickett J (2008) Identification of volatile compounds used in host location by the black bean aphid, *Aphis fabae*. *J Chem Ecol* **34**: 1153–1161
- Weißbecker B, Holighaus G, Schütz S (2004) Gas chromatography with mass spectrometric and electroantennographic detection: analysis of wood odour by direct coupling of insect olfaction and mass spectrometry. *J Chromatogr A* **1056**: 209–216
- Weston PA, Johnson DA, Burton HT, Snyder JC (1989) Trichome secretion composition, trichome densities, and spider mite resistance of ten accessions of *Lycopersicon hirsutum*. *J Am Soc Hortic Sci* **114**: 492–498
- Zhang W, McAuslane HJ, Schuster DJ (2004) Repellency of ginger oil to *Bemisia argentifolii* (Homoptera: Aleyrodidae) on tomato. *J Econ Entomol* **97**: 1310–1318