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Airborne β-caryophyllene disrupts virus–vector mutualism by priming tomato defenses

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Whiteflies pose a major threat to crops worldwide, primarily because they transmit begomoviruses with which they have evolved intricate mutualistic relationships. The mutualisms are known to exacerbate whitefly invasions and drive widespread plant virus pandemics. Yet, certain plant genotypes are able to resist both the whiteflies and the viruses and a good understanding of the underlying mechanisms could help to develop more resistant varieties. Here, we show that the viruliferous whitefly *Bemisia tabaci* induces an early and strong release of the sesquiterpene β-caryophyllene in cultivated tomato plants. This volatile functions as an airborne signal that primes neighboring conspecifics for enhanced resistance to begomoviruses, including Tomato yellow leaf curl virus and Papaya leaf curl China virus. These results challenge the view that whitefly-induced volatile emissions primarily benefit the insect vector, suggesting instead that the plant prioritizes antiviral defense over antiherbivore resistance. β-Caryophyllene exposure was also found to enhance the emission of β-Caryophyllene, methyl salicylate and β-myrcene upon whitefly attack, increasing plant attractiveness to the parasitoid *Encarsia formosa*. Using a β-caryophyllene overproducing transgenic tomato line and synthetic β-caryophyllene dispensers, we confirmed that β-caryophyllene exposure primes antipathogen defenses in tomato plants and confers improved plant fitness under sustained infestation by viruliferous whiteflies. Importantly, this defense priming is genotype-specific and limited to certain tomato cultivars, suggesting that β-caryophyllene-mediated resistance can be harnessed through selective breeding. Our findings reveal a volatile-based mechanism by which tomato plants may counteract the virus–vector mutualism, offering promising avenues for integrated pest and disease management.

herbivore-induced plant volatiles | whiteflies | begomoviruses | β-caryophyllene | airborne plant defense

Significance

Tomato plants attacked by whiteflies that carry begomoviruses release the volatile compound β-caryophyllene, which primes neighboring plants for enhanced antiviral defense. In doing so, this airborne warning signal disrupts the mutualism between the virus and its insect vector, leading to lower virus accumulation and increased attraction of parasitic wasps. Our findings suggest that the plant response, previously thought to be manipulated by the whitefly, may actually reflect a strategic defense prioritization favoring resistance to the highly damaging pathogens. This study reveals a layer of complexity in volatile-mediated plant immunity and highlights the potential of natural plant signals for sustainable crop protection against vector-borne viral diseases.

Whiteflies are among the most destructive agricultural and horticultural pests worldwide. Whiteflies are phloem-feeders that can cause extensive harm to crops, mainly through the transmission of begomoviruses (*Geminiviridae*, *Begomovirus*). Begomoviruses are the most important group of plant viruses in tropical and subtropical agro-ecosystems (1), with more than 200 known species that are exclusively transmitted by the whitefly *Bemisia tabaci* (2). The viruses and *B. tabaci* have been shown to engage in mutualistic relationships via their shared host plants in various contexts (3). For example, begomovirus infection can suppress the emission of terpenoids, which are important plant defensive compounds against whiteflies (4), or they may enhance the quality of the host plant as a resource for *B. tabaci*, thereby facilitating *B. tabaci* development and population growth (3, 5–7). Importantly, begomovirus infection can induce the emission of characteristic volatile blends that attract nonviruliferous *B. tabaci* while repelling viruliferous individuals, thereby promoting virus spread (8), and they may even manipulate the whiteflies' olfactory receptors to further facilitate their transmission (9). In general, volatile emissions that influence interactions with vector insects are frequent targets for manipulation by viruses (10, 11). This is attributed to the crucial role that plant volatiles play in host location by insects (12, 13) and the relatively flexible nature of volatile chemistry, which can be readily modified by pathogens compared to other plant traits (8, 11).

Plants have evolved a variety of defense mechanisms to protect themselves against their many microbial and herbivorous aggressors, including the production and emission of volatiles with specific ecological functions. In addition to their well-documented roles in direct or indirect defenses against herbivores and pathogens, plant volatiles also play a crucial role in plant–plant interactions (14–16). These volatiles can be perceived by neighboring plants, priming them to mount a stronger or faster defense response upon subsequent attacks (17–23). It is now clear that volatile-mediated plant–plant interactions are widespread (24) and that the volatile compounds responsible for priming have been

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identified in a handful of systems (25–28). Moreover, this priming of defenses can be specific to the type of the aggressor. For instance, herbivore-induced volatiles prime plants specifically for defenses against herbivores (29), whereas volatiles triggered by pathogen attack prime plants for enhanced resistance to particular microbes (30, 31). However, how plants respond to volatiles emitted by neighbors simultaneously attacked by more than one aggressor remains largely unexplored. Although rare, a few studies have offered some hints that plants might be able to distinguish volatile blends emitted from neighbors that were simultaneously attacked by viruses and their vectors, thereby priming for appropriate defenses against both (32). Aphid infestation, for instance, induces plants to emit volatile compounds, with methyl salicylate (MeSA) being a predominant component (29, 33). MeSA has been implicated in plant defense against aphids by attracting predators (34) or reducing the survival of these insects (35). MeSA has also been shown to mediate airborne defense against cucumber mosaic virus transmitted by aphids (32). These studies have revealed the complexity of plant responses to simultaneous threats and opened up new avenues for further research.

We previously found that volatiles released from tomato plants infested with *B. tabaci* can prime antipathogen defenses while suppressing anti-insect defenses in neighboring conspecifics, consequently accelerating the development rate of *B. tabaci* nymphs feeding on the exposed plants (28). This priming of antipathogen defenses by whitefly-induced volatiles has been observed across multiple tomato varieties (28, 36). This apparent trade-off, whereby whitefly-induced volatiles suppressed anti-insect defenses while priming antipathogen responses, was previously interpreted as a manipulation by the whiteflies to enhance host suitability for their offspring (28). However, it may instead represent an adaptive prioritization by the plant response (22) that favors resistance against viral pathogens, which may impose far greater fitness costs than herbivory by *B. tabaci* (37). This alternative interpretation would suggest that the plant is not being manipulated but is in fact "doing the right thing" by reallocating defense resources toward the more damaging threat.

Tomato yellow leaf curl virus (TYLCV) is exclusively transmitted by *B. tabaci* and represents a significant threat to tomato crops worldwide (38). Papaya leaf curl China virus (PaLCuCNV) is also transmitted through *B. tabaci* and primarily a problem on papaya but also crops like tomato and tobacco (39). Both viruses cause severe symptoms such as leaf stunting, yellowing, curling, and flower abortion, indeed indicating that such viruses impose stronger selective pressures on host plants than *B. tabaci* by itself (10, 40). We therefore hypothesized that priming of antipathogen defenses through whitefly-induced volatiles is an adaptive response against *B. tabaci*-vectored begomoviruses.

To test this hypothesis, we first compared volatile blends emitted from a cultivated and a wild tomato genotype infested with either viruliferous or nonviruliferous *B. tabaci*, identifying β-caryophyllene as a potential airborne signal. We confirmed this by exposing tomato plants to β-caryophyllene emitted constitutively by transgenic tomato plants or to synthetic β-caryophyllene dispensed at physiologically relevant concentrations, subsequently comparing the plants' defense responses and volatile emissions when subjected to infestation by either viruliferous or nonviruliferous *B. tabaci*. We also evaluated the effects of β-caryophyllene exposure on virus accumulation, *B. tabaci* development, and parasitoid attraction, as well as the impact of β-caryophyllene exposure on the fitness (e.g., seed production) of tomato plants infested with viruliferous or nonviruliferous *B. tabaci* under greenhouse conditions. Finally, to establish the generality of the phenomenon, we quantified β-caryophyllene emissions from six other cultivated tomato varieties and three wild tomato accessions, and two other solanaceous species, tobacco and sweet pepper, and measured their transcript levels of antipathogen genes after exposure to β-caryophyllene. We found that β-caryophyllene was only induced by viruliferous *B. tabaci* in one wild tomato species and three varieties of cultivated tomato, while other cultivars and wild accessions showed no increase or even reduced emissions upon infestation. In tobacco and sweet pepper, no emission of β-caryophyllene was detected upon whitefly infestation. These findings imply that by selecting specific β-caryophyllene-releasing tomato genotypes it should be possible to enhance resistance against begomoviruses and improve biological control of *B. tabaci*.

Materials and Methods

Plants. Seeds of the wild tomato *Solanum pimpinellifolium* (accession LA1589), *Solanum peruvianum* (accession LA2152), *Solanum habrochaites* (accession PI134417), and *Solanum chilense* (accession LA1967) were obtained from the C.M. Rick Tomato Genetics Resource Center (Davis, CA). In addition, we obtained seeds from cultivated tomato (*Solanum lycopersicum*), cultivars Micro-Tom (hereafter, MT plants), Alisa, Moneymaker, Castlemart, Zhefen302, Zheza 809, and M82, as well, from cultivated *Nicotiana tabacum* (cv. NC89), and *Capsicum annuum* (cv. Zhongjiao 5). All experimental plants were grown from seed in 500 cm^3 pots filled with a commercial potting substrate (Fafard Growing Mix 1, Agawam, MA). The plants were kept in a climate-controlled chamber (25 ± 2 °C, 60 to 70% RH, 10L: 14D photoperiod) and they were used in experiments when they carried five to six fully expanded leaves.

Viruses and Agro-Inoculation of Plants. Infectious clones of TYLCV and PaLCuCNV were kindly provided by Professor Zhou Xue-ping of Zhejiang University. The TYLCV clone was introduced into *Agrobacterium tumefaciens* strain GV3101 and subsequently infiltrated into the phloem of 6-wk-old tomato plants (cv. Mirco-Tom), following the method described by Bai et al. (41). Inoculations were performed using a 1.0 mL syringe, with injections administered to the stem or petioles of each plant. For PaLCuCNV infections, we used the same *Agrobacterium*-mediated inoculation method with the infectious clone PaLCuCNV-[CN:HeNZM1] (42). All plants were kept in an insect-free climate-controlled chamber (25 ± 2 °C, 60 to 70% RH, 10L: 14D photoperiod). Symptom development was monitored daily.

Insects. A colony of virus-free *B. tabaci* (Gennadius) MEAM1 (Hemiptera: Aleyrodidae) was reared on MT plants in a climate-controlled chamber (25 ± 2 °C, 50 to 60% RH, 10L: 14D). To produce viruliferous whiteflies, four tomato plants infected with either Tomato yellow leaf curl virus (TYLCV) or Papaya leaf curl China virus (PaLCuCNV) were placed in a ventilated cage (60 × 60 × 60 cm). Approximately 1,000 adult *B. tabaci* were transferred into the cage, and after 3 d of feeding on the virus-infected plants, the adults were collected and used as viruliferous whiteflies for subsequent experiments.

The parasitoid wasp, *Encarsia formosa*, was reared on nymphs of *B. tabaci* that were kept on tomato plants under greenhouse conditions (25 ± 2 °C, R.H. 50 to 70%, 14L:10D). The wasps were 2- to 5-d-old when used in experiments.

Plant Treatments. An individual plant was placed in a ventilated cage (20 × 20 × 40 cm), and 150 *B. tabaci* adults, either viruliferous or nonviruliferous, were then introduced into the cage. For the volatile trapping experiments, the whiteflies were allowed to feed on the plant for 1, 2, 3, 5, and 7 d. Control plants were placed in separate cages without whiteflies. Subsequent gene-expression, phytohormone, and behavioral bioassays were carried out using plant that had been infested with 50 *B. tabaci* adults, either viruliferous or nonviruliferous, for 3 d.

Volatile Trapping and Analysis. Headspace volatiles were collected following the method detailed by Zhang et al. (43). Briefly, two plants with the same treatment were placed together inside a 5-L glass jar. Purified air, filtered through silica gel, a molecular sieve, and activated charcoal, was drawn through the jar at a flow rate of 100 mL/min using a vacuum pump. Volatile compounds were trapped in a glass tube (10 cm long, 5 mm diameter) containing 50 mg of 80/100 mesh Porapak-Q (Alltech Assoc.). Teflon tubing was used to connect the air inlet,

air outlet, filter, and sampling jar. After 3 h of trapping under continuous light conditions (4,750 ± 86 lx), the trapped volatiles were eluted with 200 μL of dichloromethane, and 300 ng of nonyl acetate (Sigma) was added as an internal standard. Collections were replicated eight times for each treatment and were performed in parallel for each treatment on each experimental day. Samples were analyzed using a Shimadzu GC-2010 Plus gas chromatograph–mass spectrometer (GC-MS) equipped with an Rxi-5MS column (30 m × 0.32 mm i.d., 0.25 μm film thickness). Electron impact ionization (70 eV) was used for ionization, and mass scanning was conducted over a range of 33 to 300 m/z. The gas chromatography temperature program was as follows: an initial temperature of 40 °C (held for 3 min), followed by an increase of 6 °C/min to 220 °C (held for 5 min). Compounds were identified by comparing the mass spectra with those of authentic standards or with spectra in the NIST 08 and Wiley libraries. Relative quantification of the identified compounds was carried out by comparing their peak areas to that of the internal standard.

Exposing Plants to Synthetic β-Caryophyllene. To investigate whether β-caryophyllene plays a key role in plant–plant priming in tomato, different tomato lines were exposed to synthetic β-caryophyllene for 6 h. At the start of each experiment, two healthy tomato plants were placed inside a glass vessel (23 cm in diameter, 40 cm in height) along with either a control or a β-caryophyllene dispenser. The glass vessel was connected to an air-delivery system using Teflon tubing, and charcoal-purified air was pumped through the system at a rate of 300 mL/min, passing over the two undamaged plants during the 6-h exposure period. To ensure precise and continuous release rates of β-caryophyllene, a modified dispenser was employed, as described by von Mérey et al. (44). The dispenser consisted of a 2 mL glass vial containing 100 mg of glass wool. A volume of 5 mg of synthetic β-caryophyllene (≥ 80%; Sigma-Aldrich) was pipetted onto the glass wool and the vial was sealed with a PTFE/rubber septum. A 1 μL micropipette (Drummond, Sigma-Aldrich) was inserted through the septum, with the pipette length (3.5 cm) calibrated to release approximately 150 ng/h of β-caryophyllene, which is comparable to what is emitted by MT plants infested with 150 TYLCV-infected *B. tabaci* adults for 2 d. To assess the composition of volatiles released from the dispenser, vapor-phase compounds were collected and analyzed. β-Caryophyllene accounted for more than 92.6% of the total volatiles, while other $C_{15}H_{24}$ terpene hydrocarbons accounted for less than 7.4%, indicating that the plants were mainly exposed to β-caryophyllene and that the effect of impurities was negligible (*SI Appendix*, Fig. S1). The control dispenser consisted of an empty glass vial.

Olfactometer Tests. Responses of parasitoids to plant volatiles were tested in a Y-tube olfactometer as previously described (43). In short, air was passed through two odor sources to generate two laminar airflows in a Y-shaped glass tube. Individual wasps were released at the downwind entrance tube and their choices for either odor source were recorded. Wasps that did not make a choice within 10 min, were recorded as "no choice." Each wasp was used only once. After testing five wasps, odor sources were interchanged to avoid any influence of unforeseen asymmetries in the setup. Each pair of odors was repeated with fresh sources on 4 d with 15 wasps tested per day per treatment combination. Olfactometer assays were performed between 12.00 and 15.00 h, when wasps were most active.

Analyses of Gene Expressions and Virus Quantification by RT-qPCR. At different time points, leaf samples from different treatments were immediately frozen in liquid nitrogen and stored at −80 °C until they were used for DNA/RNA extraction. Leaf tissues from two plants were pooled as one sample. Total DNA was extracted from the leaves using the cetyltrimethyl-ammonium bromide (CTAB) method (45). Quantitative fluorescence-based RT-qPCR was used to detect TYLCV and PaLCuCNV in the total DNA samples as described by Sade et al. (46). The β-actin gene was used as reference gene to normalize the expression of TYLCV and PaLCuCNV. Total RNA was isolated using an RNA extraction kit (Axygen, Hangzhou, China), in accordance with the manufacturer's instructions. First-strand cDNA was synthesized from 200 ng RNA using a First-Strand cDNA Synthesis Kit (TaKaRa, Hangzhou, China) in accordance with the manufacturer's instructions. RT-qPCR was performed using SYBR Premix Ex Taq II (Takara, China) on an iQ5 Real-Time PCR Detection System (BIO-RAD, USA). Thermal cycling conditions were 5 min at 95 °C, followed by 40 cycles of 15 s at 95 °C, 15 s at 55 to 62 °C, and 30 s at 72 °C. The expression of defense-related genes was determined using RT-qPCR. In this case, the *GAPDH* gene was used as reference gene, and three to four biological replicates were performed per treatment. The specific primers for RT-qPCR are listed in *SI Appendix*, Table S1.

Quantification of Endogenous SA, JA, and JA-Ile. Endogenous SA, JA, and JA-Ile were extracted and quantified as described by Engelberth et al. (47) with minor modifications. Briefly, plant material (250 mg) was frozen and ground in liquid nitrogen. For quantification purposes, D6-SA (20 ng), [9,10]-dihydro-JA (15 ng) and JA-Ile-D6 (15 ng) were added as internal standards to 2 mL of 80% methanol. SA, JA, JA-Ile, and the internal standards were partitioned into an aqueous phase by centrifugation and vaporization. They were then extracted from the aqueous phase with an equal volume of ethyl acetate and then dried. Methanolysis of the extracts was achieved by adding methanol (anhydrous) and HCl (37%). Following evaporation of the solvent, the residues were esterified using an excess of ethereal diazomethane. The final samples were analyzed using gas chromatography coupled to a mass selective detector (GC–MS; 6890 N/5973 MSD, Agilent Technologies, Palo Alto, CA), equipped with an HP-5-MS column (30 m × 0.25 mm × 0.25 μm; 19091S-433, J&W Scientific, Agilent Technologies).

cDNA Cloning of *TPS12*, Vector Construction, and Plant Transformation. With the objective to transform tomato to constitutively release β-caryophyllene, the coding sequence of *TPS12* (GenBank Accession No. GU647162) was amplified by PCR from cDNA of tomato cultivar M82 using the forward primer TPS12-F and the reverse primer TPS12-R, incorporating restriction sites for *BamHI* (5′) and *SacI* (3′), respectively (48; Supporting Information *SI Appendix*, Table S1). *TPS12*, located on chromosome 6, belongs to the TPS-a clade and contains seven exons (48). It has been well documented that *TPS12* catalyzes the formation of the sesquiterpenes β-caryophyllene and α-humulene from *E*, *E*-farnesyl diphosphate in tomato (48, 49). The PCR product was subsequently cloned into the *BamHI*/*SacI* restriction sites of the binary vector pRI101, under the control of the cauliflower mosaic virus (CaMV) 35S promoter (50). The resulting vector construct was introduced into *A. tumefaciens* strain GV3101 and used to transform MT plants via vacuum infiltration (51). Transformed seeds harboring the pRI101-derived fragment were selected on agar plates containing the antibiotic kanamycin, and transformation was confirmed by PCR. Self-pollination and selection for the transgene were performed over three subsequent generations to obtain nonsegregating homozygous plants.

Effects of β-Caryophyllene Exposure on Defense Gene Expression and Viral Resistance. To test whether there is a correlation between the amount of β-caryophyllene during exposure treatment and the expression level of defense genes, healthy MT plants were exposed to volatiles from 1, 2, and 3 TPS12-1 transgenic plants (n = 4). After 6 h of exposure, TPS12-1 plants were transferred into a clean vessel, and their volatiles were collected for β-caryophyllene to confirm and quantify its emission. Simultaneously, the MT plants were subjected to infestation by TYLCV-infected whiteflies. After 3 d of infestation, leaf samples were collected, and subsequently, the transcript levels of defense-related genes *PR-1b*, *Ty1*, and *PI-I* were quantified by RT-qPCR.

To test whether β-caryophyllene emitted from TPS12-1 plants can prime neighboring MT plants for enhanced defense against viruses, healthy MT plants were exposed to volatiles from two plants of TPS12-1 plants or from two MT plants (Control; n = 4). Source and target plants were placed individually into glass vessels which were connected to a multiple air-delivery system via Teflon tubing (28). After 6 h of exposure, source and target plants were subjected to infestation by viruliferous whiteflies, or inoculated with TYLCV or PaLCuCNV, as described above.

Variation in β-Caryophyllene Emission and Priming Effects Among Tomato Genotypes. To investigate variability among tomato genotypes in viruliferous whitefly-induced β-caryophyllene emission and β-caryophyllene-mediated priming against virus infections, we collected volatiles from an additional three wild tomato species (*S. peruvianum* LA2152, *S. habrochaites* PI134417, and *S. chilense* LA1967), six cultivated tomato varieties (Alisa, Moneymaker, Zheza 809, Castlemart, Zhefen302, and M82), as well as from tobacco and pepper. We compared the amounts of β-caryophyllene emitted from plants infested for 2 d by nonviruliferous and TYLCV-infected whiteflies.

We also exposed the same plant genotypes to synthetic β-caryophyllene as described above (n = 3 to 4). We then measured the transcript levels of *PR-1b*, serving as an indicator of plant resistance against viruses, in exposed plants infested with either nonviruliferous or TYLCV-infected whiteflies.

Effects of β-Caryophyllene-Primed Defenses on Plant Performance Under Greenhouse Conditions. To assess whether β-caryophyllene-primed defenses enhance plant fitness under continuous viruliferous whitefly infestation, we conducted a trial in a greenhouse (6.5 × 6.5 m). Individual MT plants were each placed in a ventilated cage (25 × 25 × 45 cm), with a dispenser containing 10 mg of β-caryophyllene or an empty dispenser (Control) positioned just beneath the plant at about 5 cm from the lower leaves. After 24 h of exposure, 150 nonviruliferous or viruliferous *B. tabaci* adults were introduced into the cage and allowed to feed on the plant until their death. In a separate experimental trial, a single MT plant was placed in a ventilated cage (25 × 25 × 45 cm), surrounded by four MT (Control) or TPS12-1 transgenic plants positioned outside the cage. These surrounding plants were not removed until the experiment was completed. After 24 h of exposure, 150 nonviruliferous or viruliferous *B. tabaci* adults were introduced into the cage and allowed to feed on the plant until their death. To eliminate the effect of pollination efficiency on seed number and development, flowers were hand-pollinated. Seeds were collected from maturing fruits starting at 25 d postanthesis (dpa) until 35 dpa. Mature seeds were rinsed with tap water for 10 min, then dried on paper towels at room temperature for 2 d. The total number of seeds from each plant was counted. Each experiment was repeated twice, with ten plants tested per treatment per trial.

Statistical Analysis. Differences between two groups were analyzed using Student's *t* tests, whereas comparisons among three groups were assessed using one-way ANOVA followed by Tukey's multiple comparisons test. These tests were applied to data on volatile emissions, gene expression, phytohormone levels, and seed production. Normality of error and homogeneity of variance were verified in SAS according to Shapiro–Wilk and Levene tests. Binomial tests were performed to analyze the data from the Y-tube olfactometer experiments. Parasitoids that did not make a choice were excluded from the analysis. Statistical analyses were done with SAS9.1 (SAS Institute Inc., Cary, NC).

Results

Earlier Induction of β-Caryophyllene Emission from Viruliferous Whitefly-Infested Cultivated Tomato. Volatiles were collected from healthy MT or LA1589 plants and plants that had been infested by either nonviruliferous or viruliferous *B. tabaci* (150 adults for 1, 2, 3, 5, or 7 d). Gas chromatography–mass spectrometry (GC–MS) analysis detected a total of 15 major compounds that were consistently released, depending on the treatments (*SI Appendix*, Tables S2 and S3). Previous studies have shown that whiteflies typically induce significant volatile emissions from tomato plants at 5 to 7 d after infestation (28, 36) and that β-caryophyllene, methyl salicylate (MeSA), and β-myrcene serve as airborne signals mediating plant–plant interactions (28, 30, 32). We therefore focused on these three compounds. In LA1589 plants, the emission patterns of MeSA, β-myrcene, and β-caryophyllene from plants infested with TYLCV-infected *B. tabaci* were very similar to those from plants infested with nonviruliferous *B. tabaci* (Fig. 1 *A–C*). Similarly, in MT plants, MeSA and β-myrcene emissions from plants infested with viruliferous *B. tabaci* mirrored those from plants infested with nonviruliferous *B. tabaci* (Fig. 1 *F* and *G*). However, the emission of β-caryophyllene from viruliferous *B. tabaci*-infested plants was significantly higher after 2 and 3 d of infestation, compared to plants infested with nonviruliferous *B. tabaci* (2 d: $P = 0.001$; 3 d: $P = 0.032$; Fig. 1*E*), after which the emissions were again equal. We therefore hypothesized that β-caryophyllene may play a crucial role in airborne priming of plant defenses against viruses vectored by *B. tabaci*, especially because it increases early upon viruliferous whitefly infestation.

β-Caryophyllene Exposure Primes the Induction of Defenses Against Viruses in Cultivated tomato. To test this hypothesis, we first exposed MT and LA1589 plants to control or β-caryophyllene dispensers with a release rate of 150 ng h^{-1}, followed by infestation with either nonviruliferous or viruliferous *B. tabaci*. Our previous study demonstrated that β-caryophyllene alone did not trigger defense induction in tomato (28). However, upon infestation by either nonviruliferous (NW) or viruliferous (VW) *B. tabaci*, the expression of the pathogenesis-related gene *PR-1b* (52, 53) and the begomovirus resistance gene *Ty-1* (54) was significantly induced, whereas the JA-regulated defense gene *PI-I* was significantly suppressed in β-caryophyllene-exposed MT plants as compared to control MT plants (Fig. 2 *A–C*). Furthermore, β-caryophyllene exposure primed the accumulation of endogenous SA while suppressing the levels of endogenous JA and JA-Ile in MT plants infested with either nonviruliferous or viruliferous *B. tabaci* (Fig. 2 *D–F*). Notably, the expression levels of *PR-1b* and *Ty-1*, as well as the accumulation of SA, were significantly higher in caryophyllene-exposed MT plants infested with viruliferous *B. tabaci* compared to caryophyllene-exposed MT plants infested with nonviruliferous *B. tabaci* (Fig. 2 *A*, *B*, and *D*), suggesting that β-caryophyllene-primed defenses are linked to the presence of the virus vectored by *B. tabaci*.

We also measured the amounts of MeSA, β-myrcene, and β-caryophyllene emitted from exposed MT plants infested with either nonviruliferous or viruliferous *B. tabaci*. β-caryophyllene exposure primed an enhanced emission of all three volatile compounds from plants infested with viruliferous *B. tabaci* (Fig. 2 *G–I*). Furthermore, the amounts of β-caryophyllene and MeSA emitted from plants infested with viruliferous *B. tabaci* were significantly higher than those from plants infested with nonviruliferous *B. tabaci* (Fig. 2 *G* and *H*). In contrast, in infested LA1589 plants, β-caryophyllene exposure did not prime the induction of defense-related genes, the accumulation of endogenous phytohormones, or the enhanced emission of any volatile compounds (*SI Appendix*, Fig. S2).

β-Caryophyllene Exposure Primes Indirect but Suppresses Direct Defense Against Whiteflies. As β-caryophyllene exposure was found to prime the suppression of JA defenses, we evaluated how this might affect the developmental performance of viruliferous *B. tabaci* on control and β-caryophyllene-exposed MT plants. Survival rate and fecundity of viruliferous *B. tabaci* feeding on β-caryophyllene-exposed plants were significantly higher compared to those feeding on control plants (*SI Appendix*, Fig. S3).

Previous studies have demonstrated that compounds such as MeSA, β-myrcene, and β-caryophyllene can also act as indirect plant defenses by attracting natural enemies of herbivores in various plant species (55–58). Therefore, we further investigated the behavioral response of the parasitoid *E. formosa* to volatiles emitted from both control and β-caryophyllene-exposed MT plants infested with either nonviruliferous or TYLCV-infected *B. tabaci*. In a dual-choice assay, *E. formosa* showed no significant preference when offered volatiles emitted from control plants infested with nonviruliferous *B. tabaci* and those from control plants infested with viruliferous *B. tabaci* (Fig. 3*A*). However, the wasps exhibited a significant preference for volatiles from β-caryophyllene-exposed plants infested with nonviruliferous *B. tabaci* over those from control plants similarly infested (binomial test, $P = 0.038$; Fig. 3*B*). Moreover, *E. formosa* displayed a marked preference for volatiles from exposed plants infested with viruliferous *B. tabaci* compared to either control plants infested with viruliferous *B. tabaci* or exposed plants infested with nonviruliferous *B. tabaci* (Fig. 3). These findings suggest that β-caryophyllene exposure primes plants for enhanced indirect defenses (enhanced emissions of parasitoid attractants) against both nonviruliferous and viruliferous whiteflies.

Transgenic Tomato Constitutively Emitting β-Caryophyllene Shows Stronger Resistance Against Viruses Vectored by Whitefly. In *S. lycopersicum*, β-caryophyllene is synthesized by terpene synthase 12 (*TPS12*), a sesquiterpene synthase that produces both

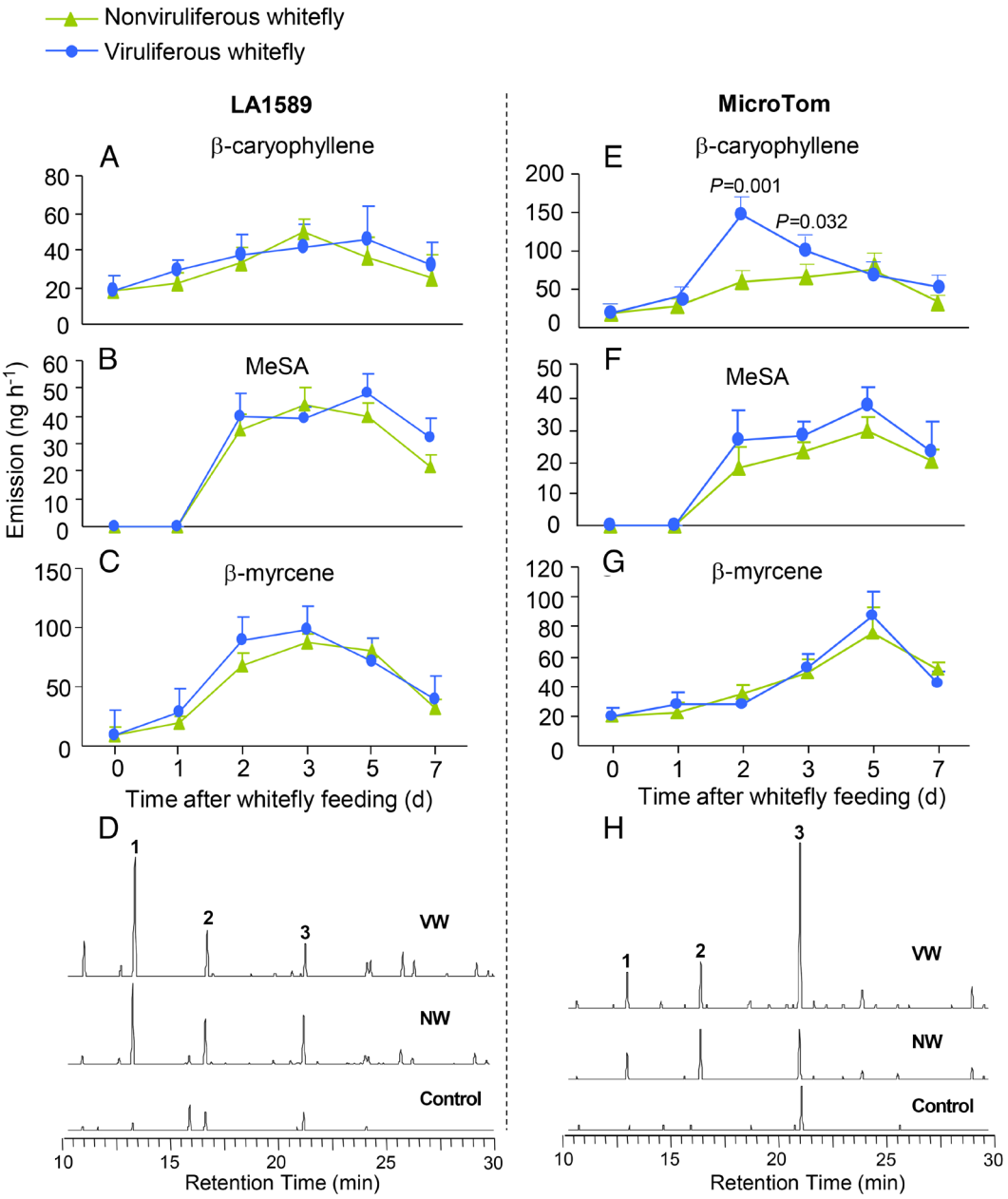

Fig. 1. Amounts of major volatile compounds released from nonviruliferous or viruliferous whitefly-infested LA1589 and MicroTom (MT) plants. Comparison of β-caryophyllene (*A*), methyl salicylate (MeSA; *B*), and β-myrcene (*C*) emission from LA1589 plants infested with nonviruliferous or TYLCV-infected whiteflies. (*D*) Typical chromatographic traces obtained from healthy LA1589 plant (Control), and LA1589 plants infested with nonviruliferous (NW) or TYLCV-infected whiteflies (VW). Comparison of β-caryophyllene (*E*), MeSA (*F*), and β-myrcene (*G*) emission from MT plants infested with nonviruliferous whiteflies or TYLCV-infected whiteflies. (*H*) Typical chromatographic traces obtained from healthy MT plant (Control), and MT plants infested with nonviruliferous (NW) or TYLCV-infected whiteflies (VW). Asterisks represent significant differences from nonviruliferous-whitefly infested plants. Values are means ± SE (n = 8). *P*-values (*P*) are shown for Student's *t* tests comparing volatile emission between treatments. 1, β-myrcene; 2, MeSA; 3, β-caryophyllene.

β-caryophyllene and α-humulene from *E*, *E*-farnesyl diphosphate, specifically in the trichomes of leaves, but not stems (49). To further investigate the impact of exposing tomato plants to β-caryophyllene on the virulence of viruses transmitted by *B. tabaci*, we generated β-caryophyllene-overproducing *S. lycopersicum* transgenic lines by transformation with the *TPS12* gene under the control of the constitutive CaMV 35S promoter. Two transgenic lines, TPS12-1 and TPS12-2, which exhibited comparable levels of *TPS12* expression, were selected for further analysis. The transgenic line TPS12-1 exhibited a significant upregulation of *TPS12* transcript levels (Fig. 4*B*) and an increased emission of β-caryophyllene (62.6 ± 5.4 ng h^{-1}; Fig. 4 *A* and *C*). Similar results were obtained for TPS12-2 (*SI Appendix*, Fig. S4).

Next, we analyzed the changes in transcript levels of defense-related genes in viruliferous *B. tabaci*-infested plants that had been exposed to volatiles from 1, 2, or 3 TPS12-1 plants, correlating these transcript changes with β-caryophyllene dose emitted by these plants. A positive correlation was observed between the transcript levels of *PR-1b* and *Ty-1* and the dose of β-caryophyllene during exposure (Fig. 4 *D* and *E*), while a negative correlation was found between the transcript level of *PI-I* and β-caryophyllene dose (Fig. 4*F*). Next, we compared the relative accumulation of TYLCV and PaLCuCNV in MT and TPS12-1 plants infested with viruliferous *B. tabaci* or artificially inoculated with infectious viral clones. The results indicated significantly lower viral accumulation in TPS12-1 plants compared to MT plants when infested

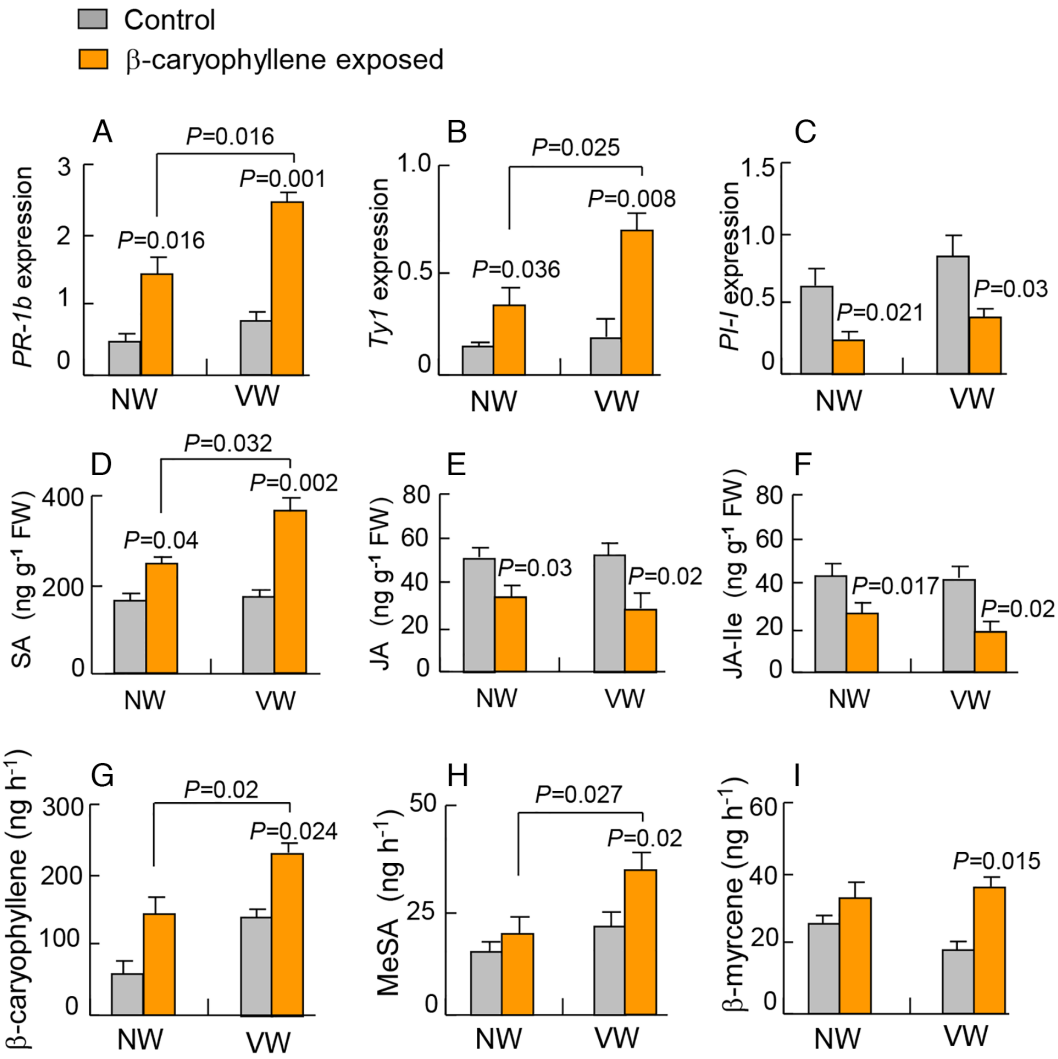

Fig. 2. Effects of β-caryophyllene exposure on defense responses induced by nonviruliferous or viruliferous whitefly infestation in MT plants. (*A–C*) Expression levels of defense genes in nonviruliferous or TYLCV-infected whitefly infested plants that had been preexposed for 6 h to a control dispenser (Control) or a β-caryophyllene-emitting dispenser (n = 3); (*D –F*) Endogenous SA, JA, and JA-Ile levels in nonviruliferous or TYLCV-infected whitefly infested plants that had been preexposed for 6 h to a control or a β-caryophyllene dispenser (n = 5); (*G–I*) Major volatile compounds emitted from nonviruliferous or TYLCV-infected whitefly infested plants that had been preexposed for 6 h to a control or a β-caryophyllene dispenser (n = 6). Error bars correspond to SEs. *P*-values (*P*) from Student's *t* tests are shown for comparisons between two groups in gene expression, phytohormone levels, and volatile emissions. NW, nonviruliferous whitefly; VW, TYLCV-infected whitefly.

by viruliferous *B. tabaci* (Fig. 4 *G* and *H*). However, no significant differences were observed between the two plant types when viruses were introduced through direct inoculation (Fig. 4 *I* and *J*). Finally, we compared the viral load of TYLCV and PaLCuCNV in MT plants preexposed to volatiles from either MT or TPS12-1 plants, following infestation by viruliferous *B. tabaci* or direct viral inoculation. Viral accumulation was significantly lower in MT plants preexposed to volatiles from TPS12-1 plants compared to those preexposed to volatiles from MT plants under *B. tabaci* infestation (Fig. 4 *K* and *L*), but no significant difference was observed between the two groups following direct viral inoculation without insect infestation (Fig. 4 *M* and *N*). Collectively, these results indicate that β-caryophyllene-primed plant defenses are closely associated with resistance to viruses transmitted by *B. tabaci* and *B. tabaci* infestation itself.

Genotype Specificity of β-Caryophyllene Emission and β-Caryophyllene-Primed Resistance Against Virus. We further analyzed the emission of β-caryophyllene from an additional three wild tomato species, six cultivated tomato varieties, tobacco, and pepper, following infestation with either nonviruliferous or TYLCV-infected *B. tabaci*. The results showed that infestation by viruliferous *B. tabaci* induced a significantly higher emission of β-caryophyllene only in one wild tomato species (*S. habrochaites* PI134417) and three cultivars (Alisa, Moneymaker, and Zheza809) at 2 d postinfestation (Table 1). In contrast, no detectable β-caryophyllene emission was observed in infested tobacco or pepper plants, regardless of whether the infestation was by infected or uninfected *B. tabaci* (Table 1). Additionally, we compared the transcript levels of *PR-1b*, a marker for plant resistance against viruses, in β-caryophyllene-exposed plants across these species. We found that β-caryophyllene exposure primed the induction of *PR-1b* in *S. habrochaites* PI134417 and four cultivated tomato species (Alisa, Moneymaker, Zheza809, and Castlemart) upon infestation by viruliferous *B. tabaci*, whereas no such induction was observed in tobacco or pepper (Table 1). In accordance, β-caryophyllene exposure suppressed the relative accumulation of TYLCV only in PI134417, Alisa, Moneymaker, Zheza809, and Castlemart upon infestation by viruliferous *B. tabaci* (*SI Appendix*, Fig. S5).

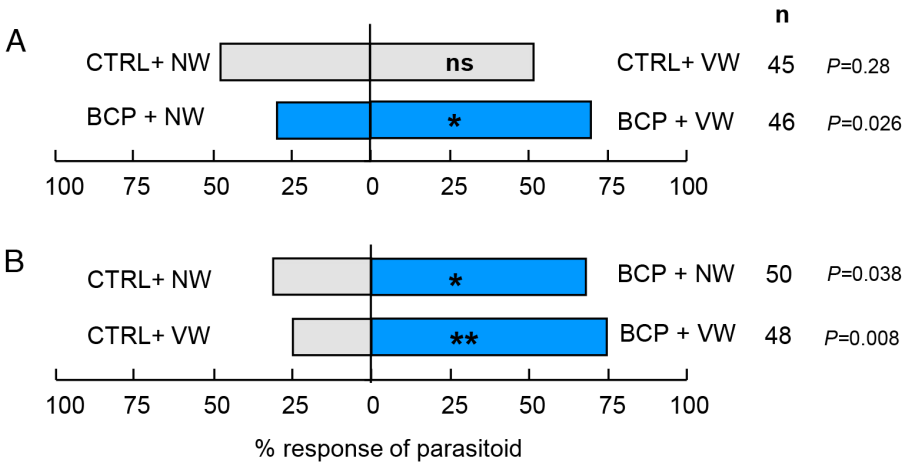

Fig. 3. Behavioral responses of female *E. formosa* wasps in a Y-tube olfactometer. Before the olfactory test, the MT plants were exposed for 6 h to either a control dispenser (CTRL, gray bar) or a β-caryophyllene-emitting dispenser (BCP, blue bar), and subsequently, were subjected to the infestation by either nonviruliferous (NW) or TYLCV-infected whiteflies (VW) for 3 d. (*A*) Effects of TYLCV infection on the attraction of *E. formosa* to *B. tabaci*-infested plants; (*B*) Effects of β-caryophyllene exposure on the attraction of *E. formosa* to *B. tabaci*-infested plants. Bars represent the percentages of wasps choosing either of the odor sources. The numbers to the right of bars represent the number of wasps making a choice. Choices between odor sources were statistically analyzed with a two-sided binomial test (*$P < 0.05$; **$P < 0.01$; ns, not significant).

β-Caryophyllene Exposure Enhances Seed Production in Tomato. Stronger induction of defense responses can impose a cost to plants, as increased investment in defense traits may limit the resources available for growth and reproduction (59). To assess the impact of β-caryophyllene exposure on reproductive fitness, we compared the seed production of control and β-caryophyllene-exposed plants under continuous infestation by either nonviruliferous or viruliferous *B. tabaci*. Under nonviruliferous *B. tabaci* infestation, β-caryophyllene-exposed plants produced a marginally lower number of seeds compared to control plants (Fig. 5*A*). However, under consistent infestation by *B. tabaci* infected with TYLCV or PaLCuCNV, seed production by β-caryophyllene-exposed plants was significantly higher than control plants (Fig. 5*A*). Similarly, preexposure to volatiles from TPS12-1 plants had no effect on seed production when plants were infested with nonviruliferous *B. tabaci*, but significantly enhanced seed production when plants were infested with viruliferous *B. tabaci* carrying either TYLCV or PaLCuCNV (Fig. 5*B*). These findings conclusively show that β-caryophyllene exposure can enhance the reproductive fitness of tomato plants, particularly under sustained pressure from viruliferous *B. tabaci* infestation.

Discussions

Airborne induction and priming of direct and indirect plant defenses against herbivores or pathogens have been widely reported across various systems (17–20, 23–32, 60). Herbivore resistance, however, is usually dependent on jasmonic acid (JA) signaling pathway (61), whereas defense against biotrophic pathogens is primarily regulated via salicylic acid (SA) signaling (62), implying that plants face trait-offs between the two types of defenses. In this study, we examined how neighboring plants respond to the volatiles emitted from plant infested with a vector insect and its associated virus. We observed an early induction of β-caryophyllene emission specifically from cultivated tomato plants infested by viruliferous *B. tabaci* (Fig. 1*E*) and found that β-caryophyllene primes antipathogen defenses upon subsequent herbivory (Fig. 2 *A*, *B*, and *D*). Importantly, this primed defense against pathogens was amplified when exposed plants were infested with viruliferous *B. tabaci* (Fig. 2 *A*, *B*, and *D*). This was confirmed in experiments with transgenic tomato plants that emitted β-caryophyllene constitutively, which in neighboring plants also enhanced plant resistance to *B. tabaci*-vectored viruses (Fig. 4 *K* and *L*). Although β-caryophyllene exposure primed the suppression antiherbivore defenses (Fig. 2 *C*, *E*, and *F*), making exposed plants more susceptible to *B. tabaci* (*SI Appendix*, Fig. S3), it also amplified the emission of β-caryophyllene, MeSA, and β-myrcene from exposed plants infested by viruliferous *B. tabaci* (Fig. 2 *G*–*I*), which increased the plants' attractiveness to a key parasitoid of *B. tabaci* (Fig. 3 and *SI Appendix*, Fig. S4). These findings reveal that β-caryophyllene functions as an airborne signal that can enhance the resistance of tomato plants to begomoviruses and bolster indirect defenses against the vector *B. tabaci*.

Previous reports and the current study show that infestation by nonviruliferous whiteflies, including *Trialeurodes vaporariorum* and *B. tabaci*, induces enhanced emission of β-caryophyllene, with a peak at 5 d postinfestation in tomato [(28, 36); Fig. 1*E*]. In comparison, β-caryophyllene emission from MT plants infested by viruliferous *B. tabaci* peaks at 2 d postinfestation, significantly earlier than in MT plants infested by nonviruliferous *B. tabaci* (Fig. 1*E*). We found this 2-d peak in β-caryophyllene emission also in one wild and three cultivated tomato species upon infestation by viruliferous *B. tabaci* (Table 1). These results suggest that β-caryophyllene may act as an airborne cue specifically signaling the risk of imminent virus infection transmitted by *B. tabaci*. This hypothesis is supported by several lines of evidence: 1) β-caryophyllene exposure resulted in stronger induction of antipathogen defenses in plants infested by viruliferous *B. tabaci* compared to those infested by nonviruliferous *B. tabaci* (Fig. 2 *A*, *B*, and *D*); 2) a positive correlation was observed between the transcript levels of antipathogen genes (*PR-1b* and *Ty-1*) and the dose of β-caryophyllene during exposure (Fig. 4 *D* and *E*); and 3) exposure to volatiles from TPS12-1 plants (with constitutive emission of β-caryophyllene) led to a significant reduction in viral accumulation in exposed MT plants infested with viruliferous *B. tabaci* (Fig. 4 *K* and *L*), but not in exposed plants artificially inoculated with viruses (Fig. 4 *M* and *N*). This lack of effect under artificial infection is likely due to the fact that, in the absence of whitefly infestation, higher β-caryophyllene emission does not prime the induction of *PR-1b* and *Ty-1* in TPS12-1 plants (*SI Appendix*, Fig. S5 *C*, *D*, *G*, and *H*), thereby failing to activate antipathogen defenses.

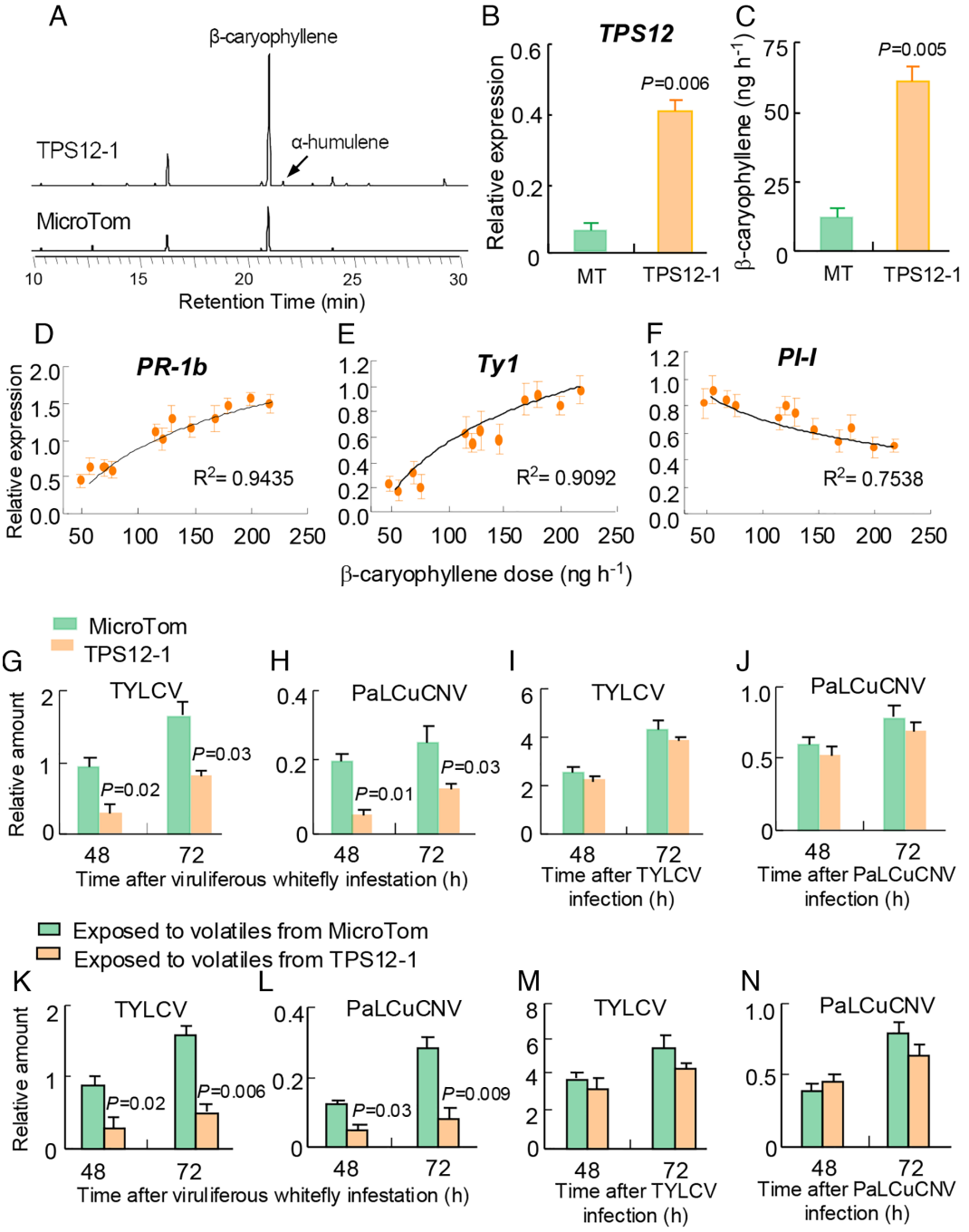

Fig. 4. Effects of β-caryophyllene emission from TPS12-1 transgenic plants on plant defense against viruses. (*A*) Typical chromatographic traces obtained from healthy MT plant and TPS12-1 plant; (*B*) Relative expression levels of *TPS12* in MT and TPS12-1 plants; (*C*) Amounts of β-caryophyllene emitted from MT and TPS12-1 plants (n = 10); (*D–F*) Correlation between the β-caryophyllene dose and transcript levels of defense genes in exposed MT plants infested with TYLCV-infected whiteflies (n = 4); (*G* and *H*) Relative amount of TYLCV or PaLCuCNV in MT and TPS12-1 plants infested by TYLCV-infected or PaLCuCNV-infected whiteflies (n = 3); (*I* and *J*) Relative amount of TYLCV or PaLCuCNV in MT and TPS12-1 plants inoculated with infectious clones of TYLCV and PaLCuCNV; (*K* and *L*) Relative amount of TYLCV or PaLCuCNV in volatile-exposed MT plants infested by TYLCV-infected or PaLCuCNV-infected whiteflies (n = 3); (*M* and *N*) Relative amount of TYLCV or PaLCuCNV in volatile-exposed MT plants agro-inoculated with infectious clones of TYLCV and PaLCuCNV (n = 3). Error bars correspond to SEs. *P*-values (*P*) from Student's *t* tests are shown for comparisons between two groups in *TPS12* expression, β-caryophyllene emission, and viral accumulation.

It should be noted that β-caryophyllene has previously been shown to exhibit antimicrobial activity against a wide range of bacteria, including species of *Bacillus*, *Pseudomonas*, and *Streptococcus* (63–66). This raises the possibility that β-caryophyllene exposure might directly affect the viruses vectored by *B. tabaci*. We did observe significant suppression of TYLCV or PaLCuCNV accumulated in infested TPS12-1 plants (Fig. 4 *G* and *H*); however, this effect is likely due to the induction by β-caryophyllene of antipathogen defenses in TPS12-1 plants (*SI Appendix*, Fig. S6). Furthermore, we did not observe any suppression effect of β-caryophyllene exposure on TYLCV or PaLCuCNV infection in *N. tabacum* (*SI Appendix*, Fig. S7), which is not primed for antipathogen defense by β-caryophyllene (Table 1). These findings suggest that β-caryophyllene does not act directly against viruses vectored by *B. tabaci* but rather enhances plant resistance to such pathogens through an indirect mechanism.

Previous research on airborne defense mediators has focused exclusively on interactions between plants and a single attacker

Table 1. Specificity of β-caryophyllene emission and β-caryophyllene-primed *PR-1b* expression under viruliferous whitefly infestation pressure

Species	Accession	As Emitter		As Receiver*	
		Emission of β-caryophyllene		Expression of *PR-1b*	
		Fold change†	*P*-value	Fold change‡	*P*-value
S. habrochaites	PI134417	1.58	0.029	1.85	**0.008**§
S. chilense	LA1967	1.26	0.067	1.27	0.075
S. peruvianum	LA2152	0.95	0.268	1.10	0.265
S. lycopersicum	Alisa	1.79	0.025	2.31	**0.006**
	Moneymaker	1.41	0.039	1.96	**0.009**
	Zheza809	1.36	0.045	1.70	**0.011**
	Castlemart	1.12	0.185	1.46	**0.032**
	Zhefen302	0.92	0.350	1.16	0.286
	M82	0.70	0.046	0.95	0.308
N. tabacum	NC89	—	—	0.93	0.435
C. annuum	Zhongjiao 5	—	—	1.16	0.350

*Plants were preexposed for 6 h to volatile β-caryophyllene (150 ng h^{-1}).
†Fold change = amount of β-caryophyllene emitted from plants infested with TYLCV-infected whiteflies/amount of β-caryophyllene emitted from plants infested with nonviruliferous whiteflies.
‡Fold change = *PR-1b* transcript levels in exposed plants infested by TYLCV-infected whiteflies/*PR-1b* levels in exposed plants infested by nonviruliferous whiteflies.
§Bold numbers indicate statistically significant differences between treatments (Student *t* test).

species (24, 30, 31). Moreover, the specificity of the information conveyed by the inducible volatile blends is closely associated with the identity of herbivore or pathogen involved (29, 31). Given that, in natural ecosystem, plants are often exposed to multiple attackers (67), it is crucial to investigate whether plants can respond to the airborne cues emitted from neighbors infested by multiple attackers, and initiate appropriate and most effective defenses. A few studies have suggested that plants might exploit airborne defenses to fend off multiple attackers. For instance, green-leaf volatiles have been shown to induce herbivore resistance (17, 68) but also enhance a plant's direct resistance to specific pathogens, particularly necrotrophic fungi (69, 70). Similarly, MeSA has been identified as an airborne signal that not only activates antiaphid defenses in neighboring tobacco plants (35) but also enhances their resistance to tobacco mosaic virus (30, 32). Despite these insights, conclusive evidence remains limited. Our study demonstrates airborne defense in the context of interactions among plants, insect vectors, and associated viruses, thereby highlighting the complexity of plant defense mechanisms in a multiattacker scenario.

Field surveys of tomato crops in Zhejiang, China, show that more than 70% of *B. tabaci* individuals are infected with TYLCV, suggesting that tomato plants are often subjected to high selective pressure from TYLCV. It appears that tomato plants mobilize defenses that specifically target begomoviruses upon detecting volatiles from neighbors infested by *B. tabaci*, whether viruliferous or nonviruliferous. Indeed, volatiles induced in tomato by *B. tabaci* infestation but also by the greenhouse whitefly *T. vaporariorum* prime antipathogen defenses in neighboring conspecifics (28, 36). These findings call into question the assumption that whiteflies manipulate their host plants. While the plant responses undoubtedly benefit the whiteflies and their offspring (28, 71, 72), our results imply that at least part of the response may represent a strategy by the plant to prioritize antiviral defenses. Considering that begomoviruses inflict more severe and lasting damage than whitefly herbivory alone (3, 10, 37), this prioritization could reflect an evolved plant response to maximize fitness under dual attack.

In the present study, we also observed that *B. tabaci*-induced β-caryophyllene primes antipathogen defenses in other four cultivated tomato species (Table 1). These findings indicate that β-caryophyllene-mediated priming is common across tomato varieties. Yet, we only observed β-caryophyllene-mediated priming defenses against pathogens in one (*S. habrochaite*, PI134417) out of four wild tomato species (*SI Appendix*, Fig. S2 and Table 1). Given that wild tomato species typically exhibit stronger constitutive resistance to begomoviruses (46), they may reduce their investment in priming defenses against viral pathogens (73). β-Caryophyllene did not prime defenses in tobacco and pepper, even though they are also hosts for TYLCV and PaLCuCNV, as well as *B. tabaci* (Table 1). Possibly, these plants utilize alternative volatile compounds as airborne signals to prime defenses. Nonanal, for instance, a compound released from whitefly-infested bean plants (74), is known to prime Lima bean defenses against the bacterial pathogen *Pseudomonas syringae* (31).

Although volatile-mediated plant–plant interactions are well documented (24), debate persists regarding its impact on plant fitness. For example, native wild tobacco plants growing near damaged sagebrush (*Artemisia tridentata*) exhibited stronger defenses against herbivory (60) yet appears to not benefit from it (75). This may be context dependent as our data indicate; neighboring receiver plants only achieved enhanced fitness when subjected to infestation by viruliferous *B. tabaci* (Fig. 5). Moreover, upon attack by viruliferous *B. tabaci*, receiver plants increase emissions of β-caryophyllene and β-myrcene, which are attractive to the parasitoid *E. formosa* [*SI Appendix*, Fig. S8; (57)], an important biocontrol agent for the

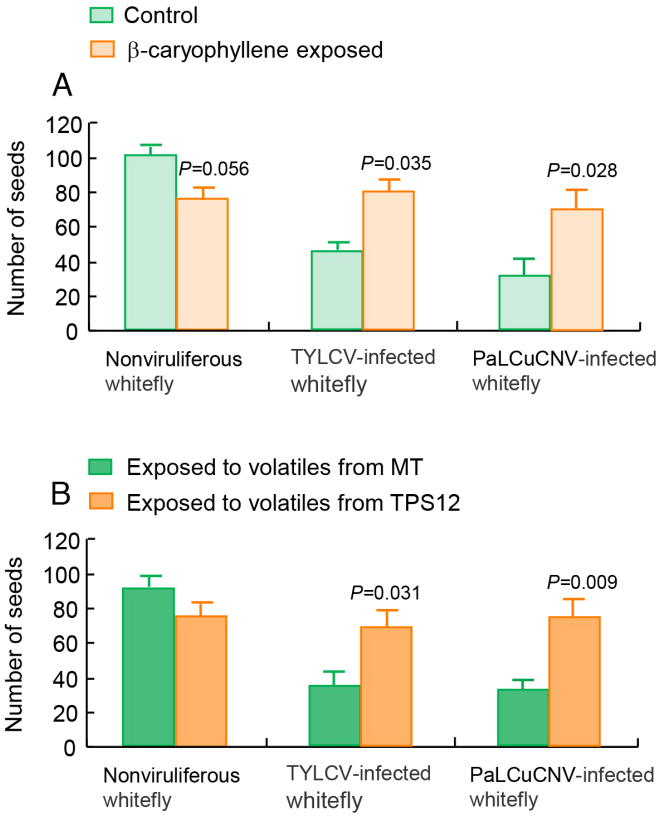

Fig. 5. Effects of β-caryophyllene exposure on seed production by MT plants under green-house conditions. (*A*) The number of seeds produced by control and β-caryophyllene-exposed MT plants under consistent whitefly infestation, either nonviruliferous or viruliferous (n = 10). (*B*) The number of seeds produced by MT plants that had been exposed to volatiles from MT or TPS2-1 plants under consistent whitefly infestation, either nonviruliferous or viruliferous (n = 10). Error bars represent SEs. *P*-values (*P*) are shown for Student's *t* tests comparing seed production between treatments.

control of whiteflies (76). This recruitment of parasitoid wasps by neighboring plants may also indirectly benefit emitter plants by reducing pest pressure within the shared environment (77–79).

Interestingly, β-caryophyllene is also an important belowground signal emitted, for instance, by maize roots upon rootworm-attack and attractive to insect-killing nematodes (80, 81). It seems that this common plant-produced sesquiterpene is an attractant to a multitude of biocontrol agents (82–84) and could be manipulated to enhance sustainable crop protection [(81), this study]. However, β-caryophyllene may also attract pests (82, 85–87) and its utilization in crop protection should carefully consider and balance the advantages and disadvantages.

The finding that β-caryophyllene functions as an airborne signal to prime defensive responses against both *B. tabaci* and its associated begomoviruses highlights a possible adaptive mechanism evolved by tomato plants to counteract the combined pressures of insect infestation and viral infection. These findings broaden our understanding of volatile-mediated plant–plant interactions in multiattacker contexts, but how the plants perceive β-caryophyllene, or any volatile compound, remains to be determined (88). In humans, β-caryophyllene is known to selectively bind to the cannabinoid receptor type 2 (CB2) and molecular docking simulations have identified a putative binding site for β-caryophyllene in the CB2 receptor (89). Although CB2 receptors are specific to animals, it is conceivable that plants may employ analogous receptor-like proteins to detect β-caryophyllene in their environment. Both forward and reverse genetic approaches might reveal candidate receptor or signaling components involved in β-caryophyllene detection in tomato. Once such receptors are identified, future research should investigate the ecological significance of β-caryophyllene-mediated priming defenses in natural settings, and evaluate its potential for application in sustainable agricultural pest management.

Data, Materials, and Software Availability. All study data are included in the article and/or *SI Appendix*.

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