

The products of a single maize sesquiterpene synthase form a volatile defense signal that attracts natural enemies of maize herbivores

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Plants can defend themselves against herbivores by attracting natural enemies of the herbivores. The cues for attraction are often complex mixtures of herbivore-induced plant volatiles, making it difficult to demonstrate the role of specific compounds. After herbivory by lepidopteran larvae, maize releases a mixture of volatiles that is highly attractive to females of various parasitic wasp species. We identified the terpene synthase TPS10 that forms (*E*)- β -farnesene, (*E*)- α -bergamotene, and other herbivory-induced sesquiterpene hydrocarbons from the substrate farnesyl diphosphate. The corresponding gene is expressed in response to herbivore attack and is regulated at the transcript level. Overexpression of *tps10* in *Arabidopsis thaliana* resulted in plants emitting high quantities of TPS10 sesquiterpene products identical to those released by maize. Using these transgenic *Arabidopsis* plants as odor sources in olfactometer assays showed that females of the parasitoid *Cotesia marginiventris* learn to exploit the TPS10 sesquiterpenes to locate their lepidopteran hosts after prior exposure to these volatiles in association with hosts. This dissection of the herbivore-induced volatile blend demonstrates that a single gene such as *tps10* can be sufficient to mediate the indirect defense of maize against herbivore attack.

parasitoid attraction | plant defense signal | terpene biosynthesis | volatile terpenes

In the last two decades, researchers have frequently observed that herbivore damage to certain plants induces the emission of volatile organic compounds that attract natural enemies of the herbivores. Termed “indirect defense,” this phenomenon has been reported in >15 different plant species to date (1–4). For example, after damage by lepidopteran larvae to maize foliage, the plant releases a complex mixture of volatiles containing a large number of mono- and sesquiterpenes, lipoxygenase pathway products, and indole (5), with the mixture varying among cultivars and between maize and related species of the Poaceae (6, 7). The high number of compounds that make up this and other herbivore-induced volatile mixtures has so far impeded the identification of the compound(s) actually responsible for signaling herbivore enemies. Attempts to dissect the volatile signal emitted by herbivore-damaged leaves of lima bean (1) and maize (8), for instance, could not identify a specific compound responsible for enemy attraction, suggesting that mixtures constitute the active signal. However, the application of individual plant volatiles, such as methyl salicylate and the sesquiterpene alcohol nerolidol, in behavioral assays have been occasionally reported to attract parasitoids (9) and predatory mites (10–12).

The volatile signal emitted by maize seedlings after attack by lepidopteran larvae attracts females of the parasitic braconid wasp *Cotesia marginiventris* (Hymenoptera), which oviposits in the larvae (5). To determine the role of specific volatiles in

attracting *C. marginiventris*, we sought to learn more about the regulation of volatile emission at the molecular level in maize to develop tools for manipulating it *in planta*. The volatiles emitted by the inbred line B73 are dominated by ≈ 60 sesquiterpene hydrocarbons, which fall into five differentially occurring groups (13). Herbivore attack induces only one of these groups, which consists of (*E*)- β -farnesene, (*E*)- α -bergamotene, and seven additional sesquiterpene hydrocarbons in a fixed relative proportion (Fig. 1 *A* and *B*). Investigations of maize terpene biosynthesis suggest that each of the five sesquiterpene groups is formed by a single multiproduct terpene synthase from farnesyl diphosphate (14). We isolated a terpene synthase, TPS10, that forms the herbivore-induced sesquiterpene volatiles of maize. With one exception, these compounds are not available commercially and are not accessible by convenient laboratory synthesis. Hence, to study the response of *C. marginiventris* to these sesquiterpenes, we overexpressed the isolated terpene synthase in *Arabidopsis thaliana*, generating plants that emit high levels of these compounds. Use of these transgenic plants in bioassays with parasitic wasps demonstrated that the sesquiterpenes produced by TPS10 can provide a volatile signal for the indirect defense of the plant against herbivore attack.

Results and Discussion

The elucidation of the functional role of individual volatiles emitted by herbivore-damaged plants has been hampered by the complexity of the blends and the difficulty of obtaining individual compounds with the correct chirality for bioassays (1, 8). We therefore attempted to identify the genes responsible for volatile biosynthesis in the hope of using these to produce the volatiles in a heterologous system. To identify the terpene synthase genes responsible for the production of the herbivore-induced terpenes, we screened a public EST database (15) for terpene synthase-like sequences. The complete cDNAs of several terpene synthases were cloned and overexpressed in a bacterial system to characterize their enzymatic activity. The enzyme encoded by the maize terpene synthase clone 10 (*tps10*) converted farnesyl diphosphate into the characteristic blend of sesquiterpenes observed after herbivory of maize with the same relative proportions of (*E*)- β -farnesene, (*E*)- α -bergamotene, and the seven minor products as those observed in the emissions from the living plant (Fig. 1 *C* and *D*). RNA hybridization analysis revealed that *tps10* transcripts were restricted to herbivore-damaged maize (Fig. 2), coinciding with the time during which the TPS10 terpene products are emitted (13). Hybridization of genomic DNA with the *tps10* cDNA and sequence comparisons with maize EST databases demonstrated that *tps10* is a single-copy gene with an amino acid sequence identity of

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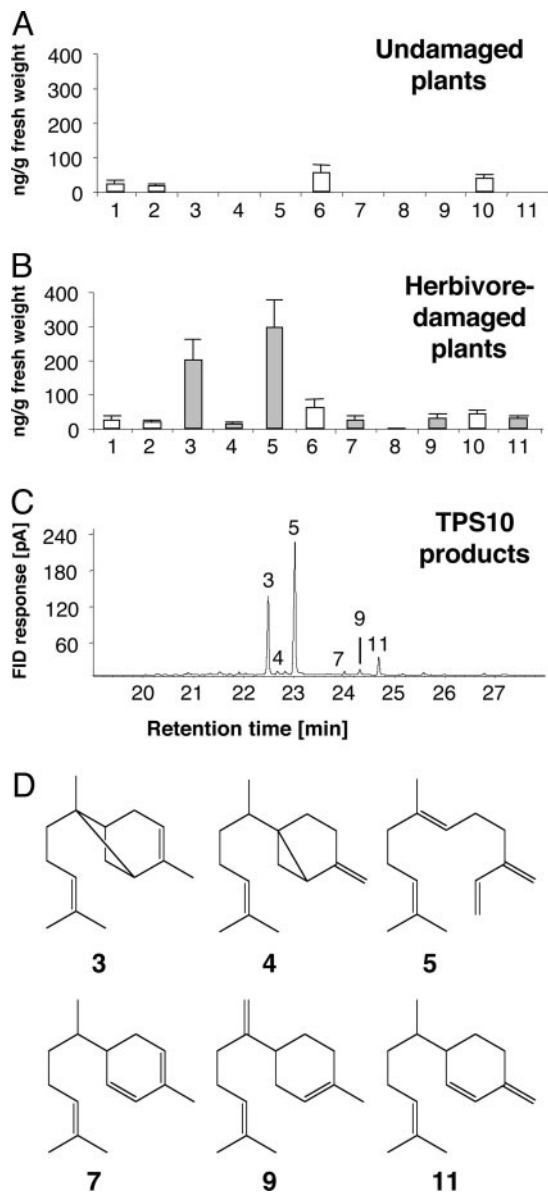


Fig. 1. The sesquiterpene hydrocarbons emitted by maize after herbivore damage are formed by the terpene synthase TPS10. Volatile sesquiterpene hydrocarbons released by intact (A) and herbivore-damaged (B) 2-week-old maize plants were collected by headspace trapping. (C) TPS10 was overexpressed in *Escherichia coli*, and products were collected after incubation with farnesyl diphosphate as substrate. FID, flame ionization detector. The sesquiterpenes identified are as follows: 1, α -copaene; 2, (*E*)- β -caryophyllene; 3, (*E*)- α -bergamotene; 4, sesquisabinene A; 5, (*E*)- β -farnesene; 6, germacrene D; 7, zingiberene; 8: α -muurolene; 9, β -bisabolene; 10, δ -cadinene; and 11, sesquiphellandrene. (D) The structures of the major sesquiterpene hydrocarbons are shown.

72% or less to all other maize terpene synthases (data not shown).

To determine whether the TPS10 sesquiterpene products indeed constitute a signal in plant indirect defense, we tested their attractiveness to *C. marginiventris*. Because laboratory synthesis of each of the chiral sesquiterpenes making up the TPS10 blend would be an arduous project, the volatile mixture was produced by overexpression of *tps10* in *A. thaliana* (*Arabidopsis*). This species is particularly suited for terpene production because it does not normally release detectable levels of volatile terpenes at the rosette stage (Fig. 3B). Plants transformed with

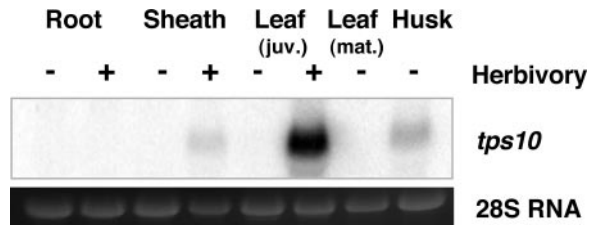


Fig. 2. High transcript levels of *tps10* are found only in herbivore-damaged maize leaves. RNA was isolated from plants of the cultivar B73 that were 2 weeks old, except for the mature leaf and husk samples, which were isolated from plants after anthesis. (Upper) The RNA was hybridized with radiolabeled *tps10* cDNA, washed, and analyzed with a PhosphorImager. (Lower) Ethidium bromide-stained agarose gel with 28S rRNA as a control for equal RNA loading.

tps10 under control of a cauliflower mosaic virus 35S RNA promoter released a sesquiterpene mixture (Fig. 3A) that is identical to that of herbivore-damaged maize (Fig. 1B) and to that of TPS10 heterologously expressed in *Escherichia coli* (Fig. 1C). In the rosette stage, the quantity of total sesquiterpenes emitted ($\approx 2.1 \mu\text{g/g}$ leaf) was comparable to that of maize plants. The transgenic *Arabidopsis* plants were used directly in bioassays with *C. marginiventris* females in a six-arm olfactometer (16, 17). The parasitic wasps were given a choice among the volatiles of *Arabidopsis* expressing TPS10, the volatiles of wild-type *Arabidopsis*, and clean air (Fig. 4A). Of all wasps tested, 69% walked into the arms carrying the odor of the *Arabidopsis* plants, whereas the remaining wasps did not make a choice or chose one of the four arms carrying clean air (Fig. 4B). Naive wasps with no prior oviposition experience showed no preference for the odor of TPS10-expressing plants over wild type ($F_{1,71} = 1.57$, $P = 0.171$), indicating that *C. marginiventris* does not exhibit an innate response to the volatiles formed by TPS10 (Fig. 4C). Indeed, females of this species are initially not attracted to these typical induced plant signals (18) but appear to exploit them as host location cues only after associating them with the presence of hosts during an initial oviposition event (19, 20). Therefore, we tested a second group of wasps that had had a previous oviposition experience in larvae of the potential host *Spodoptera littoralis* (Lepidoptera) in the presence of TPS10-expressing *Arabidopsis*. These wasps were significantly attracted to the TPS10 plants in the olfactometer (TPS10: $F_{1,71} = 10.01$, $P = 0.003$), indicating that they perceived the TPS10 sesquiterpene volatiles, associated them with the oviposition experience, and

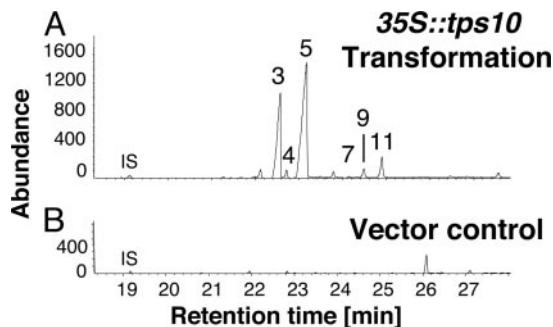


Fig. 3. *Arabidopsis* transformed with *tps10* emitted $\approx 2.1 \mu\text{g}$ per plant per h of the TPS10 sesquiterpene products. The ORF of *tps10* was cloned into the vector pBIN420 and transformed into *Arabidopsis*. The volatiles from transgenic plants (A) and control plants (B) (transformed with the same vector lacking any insert) were collected. The compounds are as follows: 3, (*E*)- α -bergamotene; 4, sesquisabinene A; 5, (*E*)- β -farnesene; 7, zingiberene; 9, β -bisabolene; and 11, sesquiphellandrene.

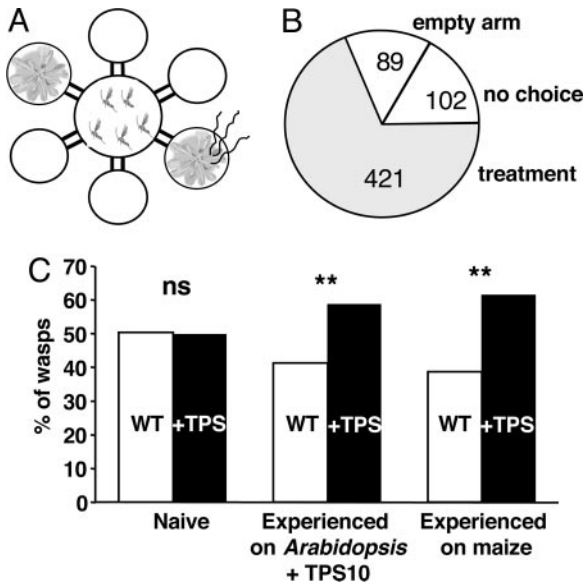


Fig. 4. Responses of the parasitic wasp *C. marginiventris* to transgenic *Arabidopsis* that emits TPS10 sesquiterpenes. Transgenic, TPS10 sesquiterpene-releasing *Arabidopsis* and wild-type plants were placed in two arms of a six-arm olfactometer (A) to test the attraction of parasitoid females (B). Three groups of parasitoids were tested (C), including naive wasps, wasps with a previous oviposition experience in a host larva in the presence of transgenic *Arabidopsis* emitting TPS10 sesquiterpenes, and wasps with a previous oviposition experience in a host larva feeding on maize. The parasitoids were tested in groups of six. **, significant preference ($P < 0.01$) for the odor of the transgenic *Arabidopsis*.

incorporated them into their host-seeking behavior. A third group of wasps had had an oviposition experience on *S. littoralis* in the presence of volatiles from herbivore-damaged maize. These wasps also chose the transgenic plants over the wild type (TPS10: $F_{1,71} = 10.4$, $P < 0.002$), confirming that this sesquiterpene blend is learned by the parasitoid for host seeking. In an additional control experiment, we compared the odor preference of naive wasps with the preference of wasps that had a previous oviposition experience in larvae while perceiving the odor of *Arabidopsis*. The wild type-experienced wasps were slightly more responsive in the bioassays that followed (fewer wasps chose empty bottles or did not choose), but they did not show any preference when they were offered the odor of *tps10* and wild-type *Arabidopsis* ($F_{1,58} = 0.56$, $P = 0.41$). This result confirms that associative learning of the TPS10 product is necessary to change the wasps' preference in favor of the odor emitted by the transformed plants.

The behavior of *C. marginiventris* in the olfactometer bioassays indicates that TPS10 sesquiterpenes can be a genuine signal in attracting these parasitoids to herbivore-damaged maize, demonstrating the function of an herbivore-induced gene, *tps10*, in indirect plant defense. The associative learning ability of *C. marginiventris* is a critical element of this tritrophic interaction, because parasitoids without oviposition experience were not significantly attracted (18). Because TPS10 products are the only sesquiterpene hydrocarbons specifically emitted after herbivory by lepidopteran larvae, they provide a very reliable cue to the parasitoid. Other hymenopteran parasitoids rely less strongly on learning and may employ more innate responses for host finding (19, 20).

In addition to the large number of sesquiterpene hydrocarbons emitted by maize, other herbivore-induced volatiles could also have a role in indirect defense of the plant. In the olfactometer experiments, the attractiveness of the odor from both the

transgenic and wild-type *Arabidopsis* as compared with clean air suggests that *Arabidopsis* foliage itself emits minor amounts of compounds that result in innate attraction of *C. marginiventris*. This general attraction may have partially masked the attractive effect of the TPS10 products. Other herbivore-induced volatiles of importance to tritrophic interactions might be sesquiterpene alcohols, homoterpenes (21, 22), oxylipins, and indole (23). The attraction of the predatory mite *Phytoseiulus persimilis* to the sesquiterpene alcohol (3*S*)-nerolidol was recently demonstrated with transgenic *Arabidopsis* overexpressing a strawberry nerolidol synthase (12). The results indicate that (3*S*)-nerolidol is a component of the volatile signal that attracts the predatory mite to spider mite-infested plants. Because this response is innate and not dependent on learning, it provides a good example of a less flexible host–predator relationship.

The introduction of a terpene synthase gene from maize resulted in strong sesquiterpene emission from *Arabidopsis*, although wild-type plants at the rosette stage release only traces of sesquiterpenes on their own (24). This overexpression represents a specific instance of heterologous production of high concentrations of sesquiterpenes by an enzyme targeted to the cytoplasm of *Arabidopsis* (25). No phenotypic changes were noted in *Arabidopsis* TPS10 transformants that would indicate depletion of sterols, phytol, carotenoids, gibberellins, or any other terpenoid products, suggesting an ample supply of the substrate farnesyl diphosphate substrate for sesquiterpene biosynthesis. The overexpression of volatile terpenes has also been demonstrated by terpene synthases targeted to the chloroplasts of mint (26) and to the mitochondria of *Arabidopsis* (12), suggesting a large degree of flexibility of terpene metabolism in plants.

This study provides an instructive example of the value of transgenic plants in dissecting ecological interactions mediated by complex volatile signals. Once the biosynthetic genes for volatile formation are known, transformed plants can be used to deliver individual volatiles or volatile mixtures for bioassays even if the compounds themselves are not conveniently accessible commercially or by laboratory synthesis. The approach has the potential to help identify any volatile signal involved in interactions among organisms. It holds special promise for advancing our understanding of plant defense and in helping to design sustainable strategies for protecting agricultural crops against insect pests (27).

Materials and Methods

Plant and Insect Material. Seeds of the maize (*Zea mays* L.) inbred line B73 were provided by KWS Seeds (Einbeck, Germany). Plants were grown in commercially available potting soil in a climate-controlled chamber with a 16-h photoperiod, 1 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of photosynthetically active radiation, a temperature cycle of 22°C/18°C (day/night), and 65% relative humidity. To rear mature plants, seedlings were transferred in large pots (35 cm in diameter) and grown in a greenhouse with 16 h supplemental lighting and a temperature cycle of 25°C during the day and 22°C at nighttime. *A. thaliana* ecotype Columbia (Col-0) was grown in 7 × 7 cm pots in a soil consisting of 2 vol of potting mix Einheitserde and 1 vol of vermiculite (Klasmann, Groß-Hesepe, Germany) with the fertilizers Osmocote and Triabon (Scotts, Nordhorn, Germany). The plants were kept in a climate chamber at 150 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of photosynthetic active radiation, at 20°C, and >50% relative humidity. Plants for transformation were grown under short-day conditions (8 h of light) for the first 3 weeks and then transferred to long-day conditions (16 h of light) for flowering. All progeny were kept under long-day conditions. Eggs of *S. littoralis* Boisduval (Lepidoptera: Noctuidae) were obtained from Aventis (Frankfurt, Germany) and were reared on an artificial wheat germ diet (Heliothis mix; Stonefly Industries, Bryan, TX) for ≈10–15 days at 22°C under

an illumination of $750 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. For the herbivory treatments, three third instar larvae were enclosed on the middle portion of 12- to 15-day-old maize plant in a cage made of two halves of a Petri dish (9 cm in diameter) with a circle cut out of each side and covered with gauze to allow for ventilation.

Plant Volatile Collection. An automated collection system (Analytical Research Systems, Gainesville, FL) based on the design of Heath and Manukian (28) was used to analyze maize headspace volatiles. In brief, the aerial portion of a potted maize plant was placed in a large glass cylinder (50 cm high \times 20-cm diameter) whose base was fitted with two adjustable blades. The blades closed around the lower part of the leaves or ears. Air that had been passed through a charcoal-infused medium for purification and moistened to a relative humidity of 65% entered the chamber from above at a rate of 5 liters/min. After sweeping over the plant material, the air exited the chamber through a collection trap, a 150 mm \times 5 mm diameter glass tube containing 75 mg Super Q (80/100 mesh; Alltech, Deerfield, IL), at the base of the chamber. Air was drawn through the trap at a rate of 1 liters/min by an automated flow controller. The remaining air escaped through the opening around the adjustable blades providing a positive pressure barrier against the entrance of ambient air. The entire volatile collection system was contained in a controlled environment chamber (Voetsch VB1014/S, Balingen, Germany) set at 25°C, 75% relative humidity, 16-h photoperiod, and $750 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of photosynthetically active radiation. All collections were performed between 10:00 a.m. and 2:00 p.m. to avoid differences due to diurnal rhythms. After the 4-h collection period, the trap was rinsed with 0.2 ml of dichloromethane containing 400 ng of nonyl acetate as an internal standard, and the sample was analyzed by GC. To collect volatiles from transgenic *Arabidopsis* plants, a more sensitive closed-loop stripping method was applied (29). The root balls were wrapped in aluminum foil, and intact plants were placed into a desiccator. Under continuous air circulation, emitted volatiles were collected for 6–12 h on activated charcoal traps. The traps were fitted into a steel column connected to the circulation pump (mini vacuum pump DZ12/16NK; Fürgut, Tannheim, Germany). Volatiles were eluted with 40 μl of dichloromethane containing 200 ng of nonyl acetate as an internal standard and analyzed by GC.

cDNA Library Construction. Ten-day-old maize plants of the cultivar B73 were subjected to herbivory by *S. littoralis* for 4 h. One gram of leaf material was ground in a mortar to a fine powder in liquid nitrogen and added to 10 ml of TRIzol Reagent (GIBCO/BRL). The mixture was homogenized with a Polytron (Kinematika, Littau, Switzerland) for 1 min and incubated for 3 min on ice. Total RNA was isolated according to the manufacturer's instructions. From $\approx 80 \mu\text{g}$ of total RNA, the mRNA was isolated by using poly(T)-coated ferromagnetic beads (Dyna, Oslo) and transcribed into cDNA for the construction of a Marathon RACE library according to the manufacturer's instructions (BD Biosciences, Palo Alto, CA).

Isolation of the Maize Terpene Synthase *tps10* cDNA. A sequence with high similarity to plant terpene synthases was identified in a BLAST search of a public EST database (The Institute of Genomic Research, www.tigr.org). To isolate the full-length cDNA, the sequence was extended toward the 5' end by the Marathon RACE procedure (BD Biosciences Clontech) with the cDNA library described above. The obtained cDNA sequence contained an ORF of 1,599 bp and was deposited in GenBank (www.ncbi.nlm.nih.gov) with the accession number AY928078 (*tps10-B73*). The full-length cDNA was cloned and sequenced twice independently.

Heterologous Expression of the Terpene Synthase Gene *tps10*. The ORF of the terpene synthase gene was cloned into the bacterial expression vector pASK-IBA7 containing a streptavidin tag (IBA, Göttingen, Germany) with the primers forward: ATGGTAACCTGCATTAGCGCATGGATGCCACCGCCTTCCAC and reverse: ATGGTAACCTGCATTATATCAGAATAATGATATTGGATCCACAAAGA. The construct was transformed into the *E. coli* strain TOP10 (Invitrogen) and fully sequenced to avoid errors introduced by DNA amplification. Liquid cultures of the bacteria harboring the expression constructs were grown at 28°C to an OD₆₀₀ of 0.5. The expression of pASK-IBA7 constructs in TOP10 cells was induced with 200 μg /liter anhydrotetracycline (IBA). After 20-h incubation at 18°C, the cells were collected by centrifugation and disrupted by 4 30-s treatments with a sonicator (UW2070; Bandelin, Berlin) in chilled extraction buffer [50 mM Mopso, pH 7.0/5 mM MgCl₂/5 mM sodium ascorbate/0.5 mM PMSF/5 mM DTT/10% (vol/vol) glycerol]. The cell fragments were removed by centrifugation at 14,000 \times g, and the supernatant was desalted into assay buffer [10 mM Mopso, pH 7.0/1 mM DTT/10% (vol/vol) glycerol] by passage through a Econopac 10DG column (Bio-Rad). The enzyme was further purified on a Strep-Tactine affinity column (IBA) according to the manufacturer's instructions. The protein concentration of the purified extract was determined by the Bradford method (34) using the BioRad reagent with BSA as standard.

Assay for Terpene Synthase Activity. The assay contained 50 μl of the bacterial extract and 50 μl of assay buffer with 10 μM (*E,E*)-farnesyl diphosphate, 10 mM MgCl₂, 0.05 mM MnCl₂, 0.2 mM NaWO₄, and 0.1 mM NaF in a Teflon-sealed, screw-capped 1 ml GC glass vial. The assay was overlaid with 100 μl of pentane to trap volatile products and incubated for 30 min at 30°C. The reaction was stopped by mixing, and 2 μl of the pentane phase was analyzed by GC.

GC and Terpene Identification. A Hewlett-Packard model 6890 gas chromatograph was used with the carrier gas He at 1 ml/min, splitless injection (injector temperature, 220°C; injection volume, 2 μl), a DB-WAX column (polyethylene glycol, 300 m \times 0.25 mm \times 0.25 μm film; J & W Scientific, Folsom, CA), and a temperature program from 40°C (3-min hold) at 5°C min⁻¹ to 240°C (3-min hold). The coupled mass spectrometer was a Hewlett-Packard model 5973 with a quadrupole mass selective detector; transfer line temperature, 230°C; source temperature, 230°C; quadrupole temperature, 150°C; ionization potential, 70 eV; and a scan range of 40–350 atomic mass units. For accurate quantification, compounds were analyzed by GC with a flame ionization detector (FID) operated at 250°C by using the conditions described above, except that the carrier gas was H₂ at 2 ml/min. Peaks were compared with that of the internal standard, assuming equal response factors. Compounds were identified by comparison of retention times and mass spectra to those of authentic standards obtained from Bedoukian (Danbury, CT), or by reference spectra in the Wiley and National Institute of Standards and Technology libraries and in the literature (30). Many standards not commercially available were obtained as described elsewhere (14). A zingiberene standard was obtained as an essential oil by a simultaneous steam distillation–pentane extraction of ginger roots (*Zingiber officinale*). All analyses were performed at least six times. Means and SEs of the FID measurements are shown.

RNA Hybridization Analysis. Plant RNA was prepared with the RNeasy plant mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. To verify an even loading of RNA, the gel was stained with ethidium bromide and visualized. The ORF of *tps10* was amplified from the pASK-IBA7 construct

with the primers forward: ATGGTAACCTGCATTAGCG-CATGGATGCCACCGCCTTCCAC and reverse: ATGGT-AACCTGCATTATATCAGAATAATGATATTGGATCCA-CAAAGA. This fragment was used as a template for synthesis of a 1,196-bp probe by linear PCR with the primer TACTT-GAAAGGCTCCCACC in the presence of adenosine [³²P]-triphosphate according to the Strip-EZ PCR procedure (Ambion, Austin, TX). Blotting on a Nytran Plus nylon membrane (Schleicher & Schuell), hybridization, and washing were carried out following standard procedures. The blots were scanned with a Storm 840 PhosphorImager (Molecular Dynamics) for quantification.

Generation of Transgenic *Arabidopsis*. The ORF of *tps10* was amplified from the pASK-IBA7 construct with the primers forward: ACTGGTACCTATGGATGCCACCGCCT and reverse: TCA-GAGCTCTCTAGAATAATGATATTGGATCC and inserted as a KpnI-SstI fragment between the 35S promoter of the cauliflower mosaic virus (CaMV) and a nopaline synthase terminator into the binary vector pBIN420 (31). The obtained construct was introduced into the *Agrobacterium tumefaciens* GV3101 strain, which was used to transform *Arabidopsis* (ecotype Col) plants by the floral dip method (32). Transgenic lines were selected by kanamycin resistance, and transformation was additionally confirmed by PCR analysis. Control lines were established carrying the empty insertion cassette only.

Olfactometer Experiments. *Zea mays* (inbred line B73) and *A. thaliana* plants were grown under conditions similar to the experiments described above. Seeds were planted in plastic tubes (4-cm diameter, 10-cm height) with fertilized potting soil (Coop, Basel, Switzerland) and transferred to a climate chamber. *Arabidopsis* plants were used when they had fully formed rosettes (≈ 5 weeks after planting), whereas maize plants were used 10 days after planting, when they had three developed leaves. Fifteen hours before an experiment, the maize plants were infested with 20 *S. littoralis* caterpillars to induce volatile emission.

S. littoralis caterpillars and the solitary endoparasitoid *C. marginiventris* were reared as described in ref. 7. *S. littoralis* eggs were supplied by Syngenta (Stein, Switzerland), and second- and third-instar larvae were used for rearing of parasitoids, infestation of plants, and oviposition experiences. Adult parasitoids were kept in plastic cages (30 × 30 × 30 cm) in incubators at 25 ± 1°C and provided with moist cotton wool and honey. We tested mated, 2- to 4-day-old females, which were both naive and experienced individuals. Experienced females were obtained by placing them in a tube containing 20 *S. littoralis* larvae on top of a vessel that held an odor source (either a *Spodoptera*-infested maize plant or a transgenic *Arabidopsis* plant). The wasps were

released in the tube one by one and removed after 3–5 ovipositions. A nylon screen prevented the wasps from entering the vessels but allowed them to perceive the odor of the plant below them. For each replicate, six wasps were provided with this oviposition experience, and subsequently the procedure was repeated with 20 fresh larvae and six new wasps.

The attractiveness of the *Arabidopsis* to *C. marginiventris* females was tested in a six-arm olfactometer (19). In all experiments, a wild-type and a transgenic plant were placed in two odor-source vessels connected to opposite olfactometer arms, while the four remaining arms were connected to empty vessels. Cleaned and humidified air entered each vessel at 1.2 liter/min, carrying the volatiles via the arms to a central cylinder. Simultaneously, 0.6 liter/min of air was pulled out through volatile collection traps containing the adsorbent Super Q (see below), which were connected to a port at the top of each vessel. Wasps of the same experience type were released in groups of six into the central glass cylinder and could choose to enter one of the six arms. After entering an arm, the wasps' passage was blocked by a stainless steel mesh, and eventually they oriented toward a light source and were trapped in glass bulbs where they were counted and removed. Wasps that did not enter an arm within 30 min were considered to have made "no choice," whereas wasps choosing an arm were considered "responsive." A total of six groups of six wasps were tested during a 3-h period, alternating among the three experience types (naïve, experienced with transgenic *Arabidopsis* odor, and experienced with induced maize odor).

The behavioral responses of the parasitoids to different odor sources were analyzed with a log-linear model corrected for the expected distribution of the wasps within the olfactometer (33). "No choice" wasps were not included in the analyses. The model was fitted by maximum quasilielihood estimation in the software package R (Version 1.9.1; R-Project, Vienna), and its adequacy was assessed through likelihood ratio statistics and examination of residuals. To confirm the emissions of the expected volatiles from the experimental *Arabidopsis* plants, volatiles were collected during the assays on Super Q traps (25 mg, 80–100 mesh; Alltech) connected to flowmeters (Analytical Research System) and a vacuum pump. Air carrying the volatiles was pulled through each trap for 3 h at a rate of 0.6 liter/min during each behavioral bioassay. The traps were extracted and analyzed as described above.

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