Supporting Information

Bleeker et al. 10.1073/pnas.1208756109

SI Materials and Methods

Plant Material. Tomato (*Solanum habrochaites* PI127826) seeds were obtained from Keygene N.V. and grown in a greenhouse (18–23 °C and a 16-h light). For inhibitor studies cuttings were placed in a hydroponics system in a climatized room (8/16 h light/dark, 21 °C, 70% relative humidity). Six cuttings were grown in the presence of 10 μM fosmidomycin (Invitrogen), compared with six untreated plants. After 5 wk, the chlorophyll contents of the treated cuttings severely decreased and leaves, as well of those of control plants, were taken and analyzed. An interspecific cross between *Solanum lycopersicum* cv Moneymaker and *S. habrochaites* accession PI127826 was made and F2 plants were screened for the presence of zingiberene in leaf material as described below. Cuttings of F2 plants were used in bioassays.

Expression Analyses. Trichomes were isolated by shaking stems in liquid nitrogen and, with the remaining bald stem material, used for cDNA synthesis, along with the other tissues. Additionally, RNA was isolated from *S. habrochaites* 24 h after spraying with either 1 mM jasmonic acid (Ducheva Biochemicals) in tap water containing 0.05% SilwetL-77, or water with SilwetL-77.

RNA from 4-wk-old-plants was isolated using the Qiagen RNeasy plant Mini kit according to the manufacturer's instructions and DNase treated (TURBO DNase kit, Ambion). cDNA was synthesized from 1 μg of total RNA using the RevertAid kit (Fermentas). For transcript abundance analyses cDNA equivalents of 10 ng RNA were used with the SYBR Green Real-Time PCR Mastermix (Invitrogen) and 300 nM of each primer and dispersed on a 96-well plate in the ABI 7500 Real-Time PCR System (Applied Biosystems). Specificity was verified by dissociation analysis. Expression of the tomato RUB1 conjugating enzyme (RCE1) was used for normalization (GenBank accession no. AY004247). Primer efficiencies were calculated by analysis of amplification curves of a cDNA dilution range. Three biological replicates were analyzed individually. Primers: zFPSf: 5′-GCA-AAG-GAT-AAG-GGT-TTA-GAC-GTA-TCC-3′, zFSr: 5′-TCA-AGA-AAT-CAA-CCT-CCC-CCT-TGC-3′, ShZISf: 5′-GCA-TTA-CAG-AAT-GAG-TTC-ACG-AG-3′, ShZISr: 5′-AAG-TGT-ATC-AAC-CAA-GCA-AAG-C-3′, RCE1f: 5′-GAT-TCT-CTC-TCA-TCA-ATC-ATT-CG-3′, RCE1r: 5′-TTT-GGG-GAC-ATC-TTC-GGA-TGA-A-3′.

GC-MS Analyses. Leaves were washed in 5 mL of hexane for 5 min and dried with Na_2SO_4. Terpenoids were analyzed by injection of 1–3 μL into the Optic injection port (ATAS GL international) at 50 °C, subsequently heated to 275 °C (4 °C·s^{-1}) followed by gas-chromatography/mass-spectrometry. The split flow was 0 mL for 2 min and then 25 mL·min^{-1}. For total-tissue analyses, leaf material was ground in liquid N_2, and taken up in saturated $CaCl_2$. Tissue and enzyme products were sampled with a Solid Phase Micro Extraction fiber (SPME; 100 μm PDMS fused silica, Supelco) for 10 min after agitation and heating to 50 °C. The fiber was desorbed 1 min in the Optic injector port at 250 °C. Compounds were separated on a DB-5 column (10 m × 180 μm, 0.18 μm; Hewlett Packard) in a 6890 N GC (Agilent) with 40 °C for 1.5 min, ramp to 250 °C at 30 °C·min^{-1} and 250 °C for an additional 2.5 min. Helium was used as carrier gas, and the column flow was 3 mL·min^{-1} for 2 min and 1.5 mL·min^{-1} thereafter. Mass spectra were generated with the ion source at −70 V, 200 °C and collected with a Time-of-Flight MS (Leco, Pegasus III) at 1,850 V, making 20 scans·s^{-1}. Terpenoids were identified using standards and comparing ions spectra, retention time, and Kovats Index (1).

To separate zingiberene stereo-isoforms, the Astec CHIRALDEX B-DM column (30 m × 0.25 mm × 0.12 μm; Supelco) was selected. The program was set to 115 °C for 3 min, increased to 140 °C by 4 °C·min^{-1} where it was kept for 1 min, after which the temperature increased (2 °C·min^{-1}) to 166 °C where it was kept for 5 min before increase (40 °C·min^{-1}) to 220 °C. The injector temperature was kept at 220 °C, and column flow was kept at 1 mL·min^{-1}.

Enzyme Characterization. The candidate-sesquiterpene synthase from *S. habrochaites* PI127826 was amplified from trichome-enriched cDNA. After sequence verification the full-length cDNA was ligated into the pGEX-KG expression vector (2). The lemon-basil zingiberene synthase (3) was kindly provided by E. Pichersky (University of Michigan, MI). Constructs were transformed to C41 (DE3) electro competent *Escherichia coli* cells (4). An overnight culture was inoculated in Terrific Broth containing 100 μg·mL^{-1} ampicillin. The culture was grown to OD_{600} 0.5–0.6 at 37 °C, after which it was placed at 4 °C for 30 min. Expression was induced with 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) (Roche). After incubation (200 rpm, 16 °C, 16 h) cells were harvested (1,614 × *g*, 4 °C, 15 min). The pellet was resuspended in 2 mL assay buffer [AB; 25 mM Hepes (pH 7.2), 10 mM $MgCl_2$, 10% (vol/vol) glycerol] with lysozyme (1 mg·mL^{-1}) and Proteinase Inhibitor Mixture Complete (PICC, Roche). Cells were incubated on ice for 30 min and sonicated. Lysates were centrifuged (12,000 × *g*, 4 °C, 25 min) and stored at −80 °C. Activity assays were performed with 50 μL of bacterial lysate in 20-mL glass vials in a total volume of 500 μL AB supplemented with 5 mM DTT and either 2 μM Z,Z-FPP (2*Z*-6*Z*-farnesyl diphosphate), *E,E*-FPP (*E*-*E*-farnesyl diphosphate) GPP (geranyl diphosphate) NDP (neryl diphosphate) or GGPP (geranylgeranyl diphosphate) (Echelon Biosciences). Vials were closed with a Teflon-lined crimp cap and incubated (180 rpm, 30 °C, 1 h), after which headspace products were sampled by SPME.

To purify the GST-ZIS fusion protein, 250 mL of bacterial culture was grown and induced as described above. Cells were resuspended in 15 mL of extraction buffer (20 mM Tris·HCl, pH 7.5/100 mM NaCl/5 mM EDTA/1 mM EGTA/5 mM DTT) supplemented with 0.05% Tween 20 and 1× PICC. The recombinant protein was purified using glutathione Sepharose 4B resin (GE Healthcare, www.gelifesciences.com).

For kinetic analyses, 10 ng of purified recombinant protein was incubated with *Z,Z*-FPP (1–10 μM), in 500 μL of AB supplemented with 5 mM DTT. The mixtures were overlaid with 500 μL of hexane and incubated for 1 h at 30 °C. The reaction was stopped by vigorous mixing and centrifugation. The organic phase was collected followed by a second extraction with 500 μL of hexane. The combined extracts were dried with Na_2SO_4 injected on the GC-MS. Three replicates were performed for each *Z,Z*-FPP concentration. The K_m and K_{cat} values were determined from Lineweaver–Burk plots using Kaleidagraph software (Synergy Software).

***In Planta* Engineering.** For trichome specific expression of *zFPS* and *ShZIS* we used the tomato Methyl Ketone Synthase (MKS1) promoter (5) for *zFPS* and the Monoterpene Synthase 1 (MTS1, AY840091) (6) for *ShZIS*. To establish that expression under these promoters was trichome specific, constructs with the *uidA* reporter (GUS) driven by the MKS1 or the MTS1 promoter were constructed. The Methyl-Ketone Synthase promoter (5) was cloned from genomic DNA of the wild tomato *S. habrochaites*

PI126449. The 1733 bp fragment was used to drive GUS or *zFPS* in the pKG1662 vector of which the 35S-promoter was removed using HindIII and XbaI. A 1256 bp promoter fragment of MTS1 was obtained by performing a nested PCR using MTS1 specific primers on adapter ligated genomic DNA for *S. lycopersicum* Moneymaker. The MTS1 promoter was cloned into the pKG1662 vector driving GUS or *ShZIS* in the same way as described for MKS1. Promoter-GUS and promoter-*zFPS*/*ShZIS*-terminator cassettes were cloned into pBIN-plus (7). Constructs were transformed to *Agrobacterium tumefaciens* GV3101 and used to transform tomato *S. lycopersicum* Moneymaker (8). Plants were regenerated under kanamycin selection. The presence of terpenes was analyzed by SPME on ground leaf material or by injection of hexane leaf washes into the GC-MS. The 7-epizingiberene stereoisomer was confirmed on the B-DM column.

Analysis of promoter activity was carried out using histological GUS assays. Plant material was washed in staining buffer [phosphate buffer (40 mM K_2HPO_4/10 mM KH_2PO_4)/0.2% Triton X-100, pH 7.2] and incubated overnight at 37 °C in the presence of 1 mM X-gluc (5-Bromo-4-chloro-3-indolyl β-D-glucuronide cyclohexylamine salt). Samples were destained with 70% (vol/vol) ethanol and tissue-specific expression of the promoters analyzed under a stereomicroscope.

Bioassays. A *Bemisia tabaci* (9) population was maintained in a climatised chamber (Snijders Tilburg; T 28 °C, 16-h light, RH 75%) on cucumber. F2 cuttings, *S. habrochaites* PI127826 and *S. lycopersicum* cv. Moneymaker and transgenic lines were grown in soil and kept in a climatised greenhouse compartment. Twenty adult whiteflies were anesthetized with CO_2, placed in a clipcage (2.5 cm diameter; Bioquip) and attached to a young leaflet, in such a way there was room for the whiteflies to move to both sides of the leaflet. A leaflet of the same leaf was taken for terpene analysis. After 5 d, the number of dead flies and eggs was recorded. For the spider mite assays, *Tetranychus urticae*, originally collected in the field from Spindle Tree in the Netherlands, and *Tetranychus evansi*, collected from tomato in Vicosa, Brazil (10), were reared on common garden bean in a climatised chamber (25 °C, 16-h light 150 $\mu E \cdot m^{-2} \cdot s^{-1}$, RH 60%). Before the experiments a synchronized culture of both species was started with female mites. For the assay, per mite line 60 leaf discs of 15 mm were made of Moneymaker, PI127826 and transgenic line 2 and placed on wet cotton with their adaxial side up. On each leaf disk a female mite was placed. After 4 d, the number of eggs was recorded. For the population growth experiment, 4-wk-old transgenic tomato plants expressing *zFps* and *ShZis* and producing 7-epizingiberene and control plants without 7-epizingiberene were infested with three adult *T. urticae* females with a fine brush. The next day we checked if mites were still alive and feeding. Intact plants were maintained in a greenhouse compartment under controlled conditions (26 ± 4 °C, 70 ± 10% RH and 12-h light) and the total number of adult spider mites was recorded every 3–4 d. Each treatment was replicated four times with different plants. For the choice test with *T. urticae* spider mites we joined the edges of two leaf disks (1 cm), one from control tomato and one from transgenic tomato, on wet cotton wool with an upright needle (n = 120). On top of the needle an adult female mite was placed such that it could walk down and settle on a leaf disk. These mites came from the bean culture to prevent imprinting or experience influencing the results. After two days we recorded on which disk(s) the mite had been feeding and depositing eggs.

Larvae of the tobacco hornworm (*Manduca sexta*; Lepidoptera, Sphingidae) were hatched overnight at 28 °C from eggs received from the Max Planck Institute for Chemical Ecology in Jena, Germany. For *M. sexta* caterpillar bioassays, F2 plants producing 7-epizingiberene and control F2 plants containing only monoterpenes were maintained in the greenhouse for four weeks before start of the experiment. Freshly hatched neonates were placed on leaflets of the third leaf. To measure larval performance, larvae were weighed after 4, 7, and 10 d. Colorado Potato Beetles (CPB) were reared on potato. Feeding preference experiments were performed with naïve neonates hatched from egg clusters at RT. One larva was placed in a Petri dish with two leaf discs of F2 control and F2 7-epizingiberene producing plants on wetted filter paper. Subsequently, the percentage of tissue damage was estimated after 48 h (n = 15 replicates).

Statistics. Statistical analyses were performed using ANOVA followed by the LSD test for comparison of individual means using the statistical program SPSS (IMB SPSS Inc. 2010). Levene's test of equality of error variances was included and where necessary values were log-transformed before analyses.

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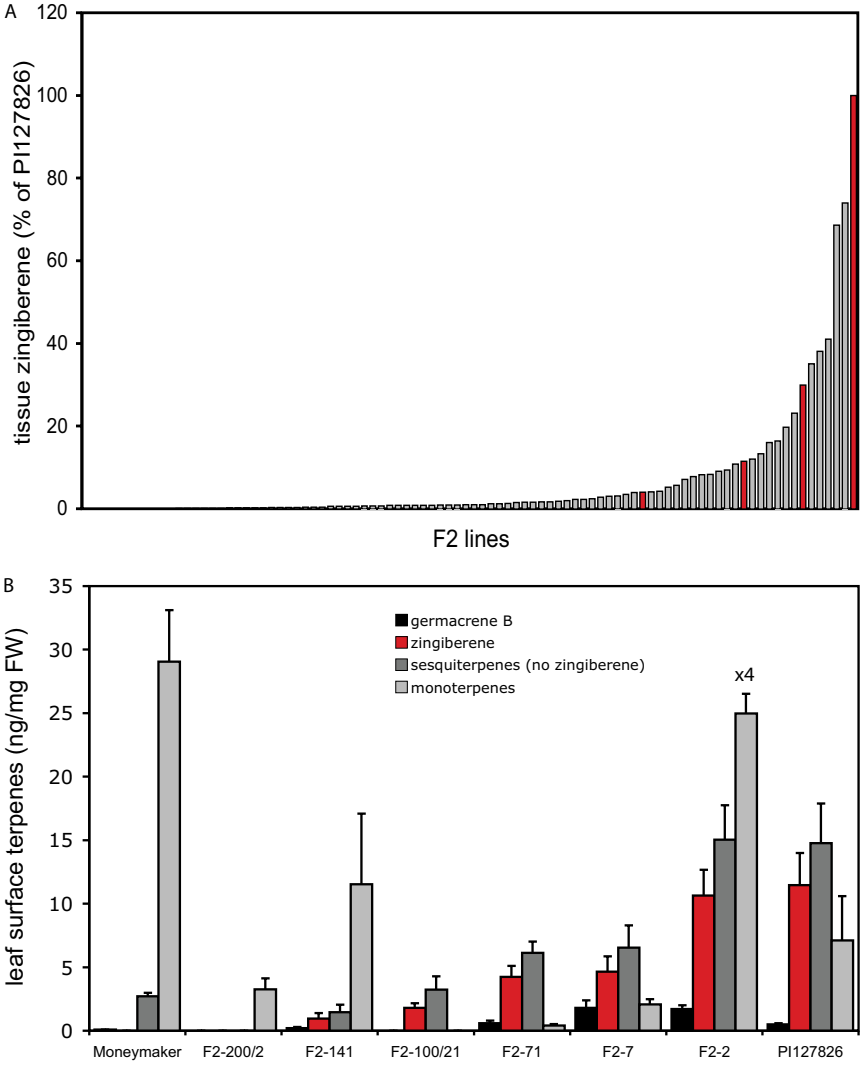

Fig. S1. (*A*) Relative zingiberene content in leaves (%) of an F2 population of *S. lycopersicum* cv Moneymaker × *S. habrochaites* PI127826. Colored bars are zingiberene levels in selected F2 plants 141, 7, 71 and 2. Zingiberene is absent in *S. lycopersicum* and 100% in *S. habrochaites* PI127826. Zingiberene was measured in ground leaves by SPME. (*B*) Terpenes produced by F2 plants of an interspecific cross between *S. lycopersicum* cultivar Moneymaker and *S. habrochaites* PI127826. Germacrene B (black bars), zingiberene (red bars), total sesquiterpenes (dark gray bars), total monoterpenes (light gray bars) (ng/mg leaf FW), measured in a leaf wash of F2 plants and both parents (n = 5–8). Bars represent means ± SE. Note: monoterpene levels in F2-2 are 4 times higher than presented.

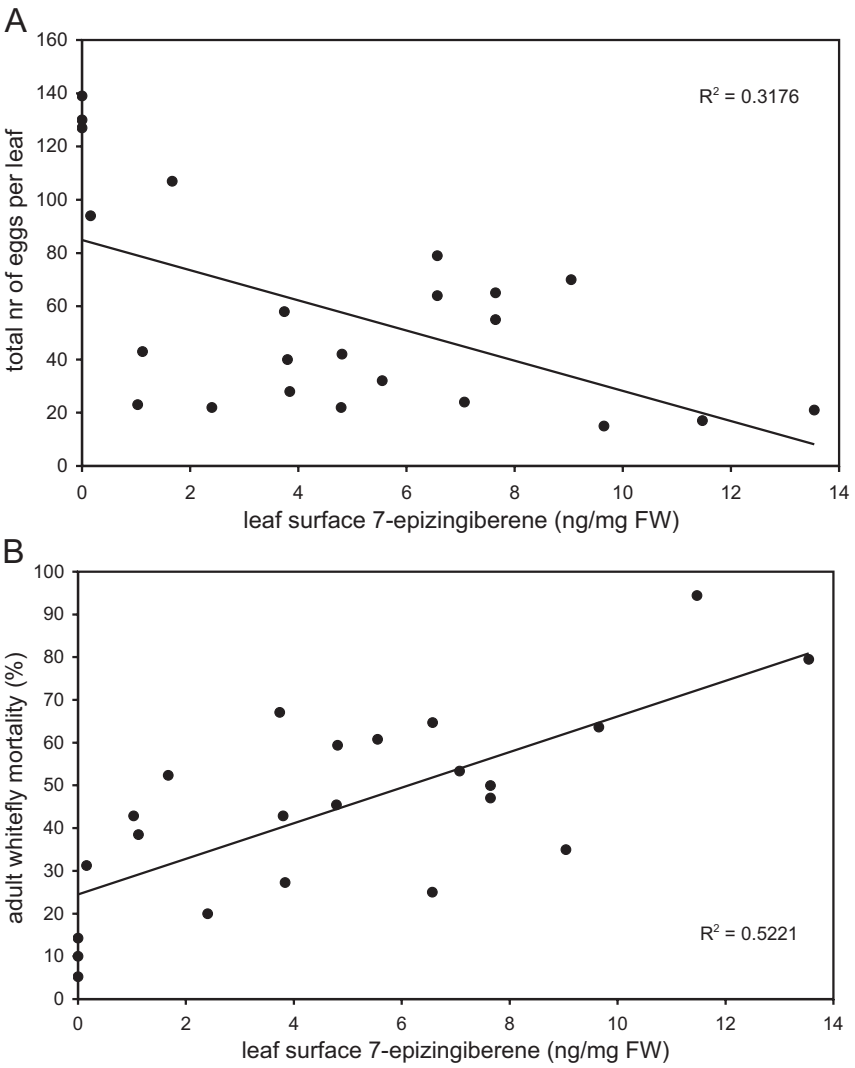

Fig. S2. Toxicity of zingiberene to *B. tabaci* in F2 plants of an interspecific cross between *S. lycopersicum* cultivar Moneymaker and *S. habrochaites* PI127826 using clip cage assays. Correlation between 7-epizingiberene (ng/mg FW) in leaf washes in F2 plants with the total number of eggs on a leaf (*A*) and mortality (%) of adult whiteflies (*B*).

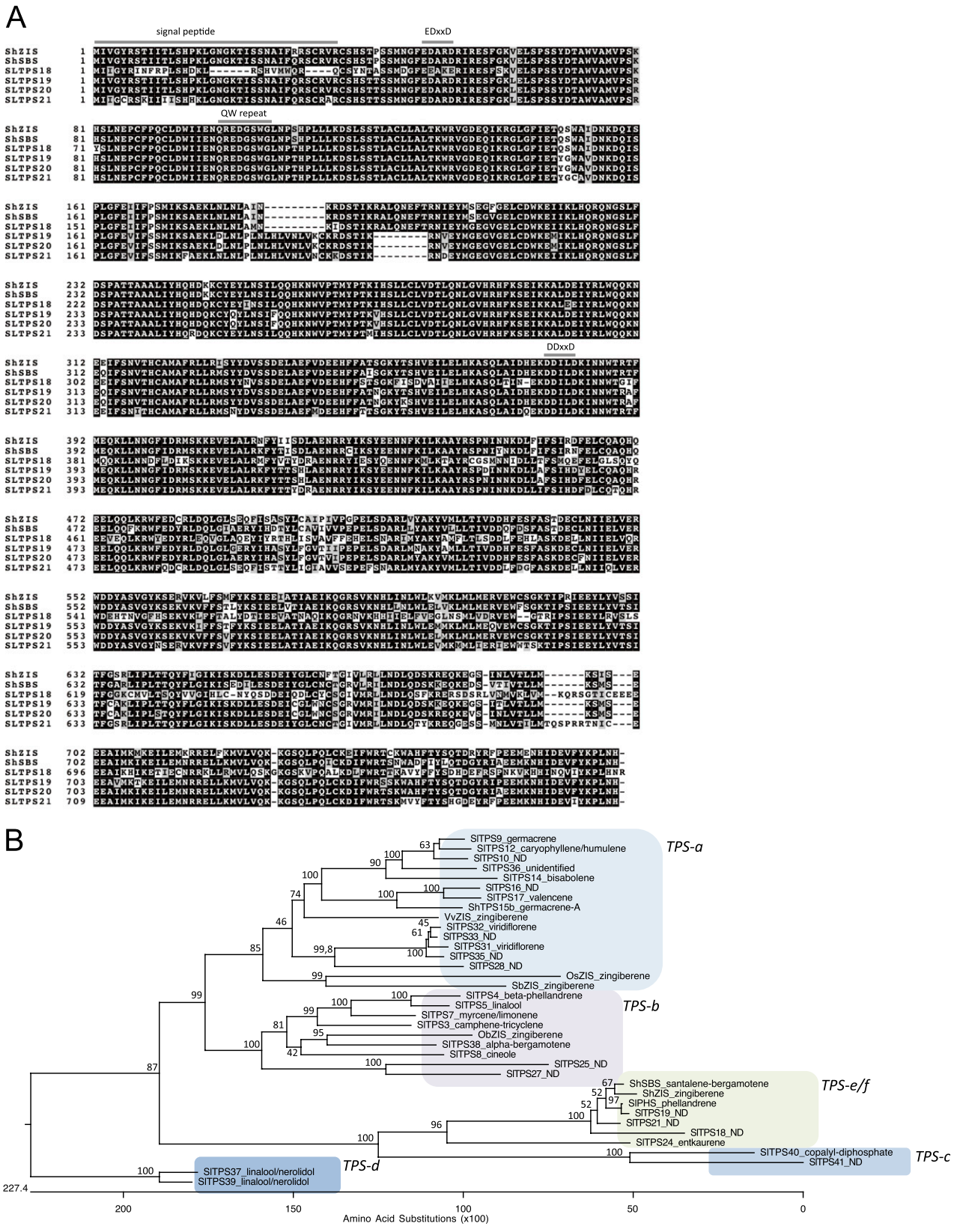

Fig. S3. (*A*) Alignment of tomato TPS class-e/f enzymes from *S.lycopersicum*, ShZIS from *S.habrochaites* PI127826, ShSBS from *S.habrochaites* LA1777. Alignments were made with CLUSTALW using the BLOSUM matrix (www.ch.embnet.org/software/ClustalW.html). Homology was visualized using Boxshade (www.ch.embnet.org/software/BOX_form.html). (*B*) Phylogenetic tree of (potentially) functional terpene synthase cDNAs of *S. lycopersicum* (Sl) and *S. habrochaites* (Sh) and zingiberene synthases (ZIS) of sorgum (Sb), rice (Os), grape (Vv), and basil (Ob). ND: activity not determined, Unidentified: product not identified. Alignments were made with CLUSTALW. The phylogenetic tree was constructed after bootstrap analysis (n = 1,000) using Lasergene DNAstar Megalign software (DNASTAR).

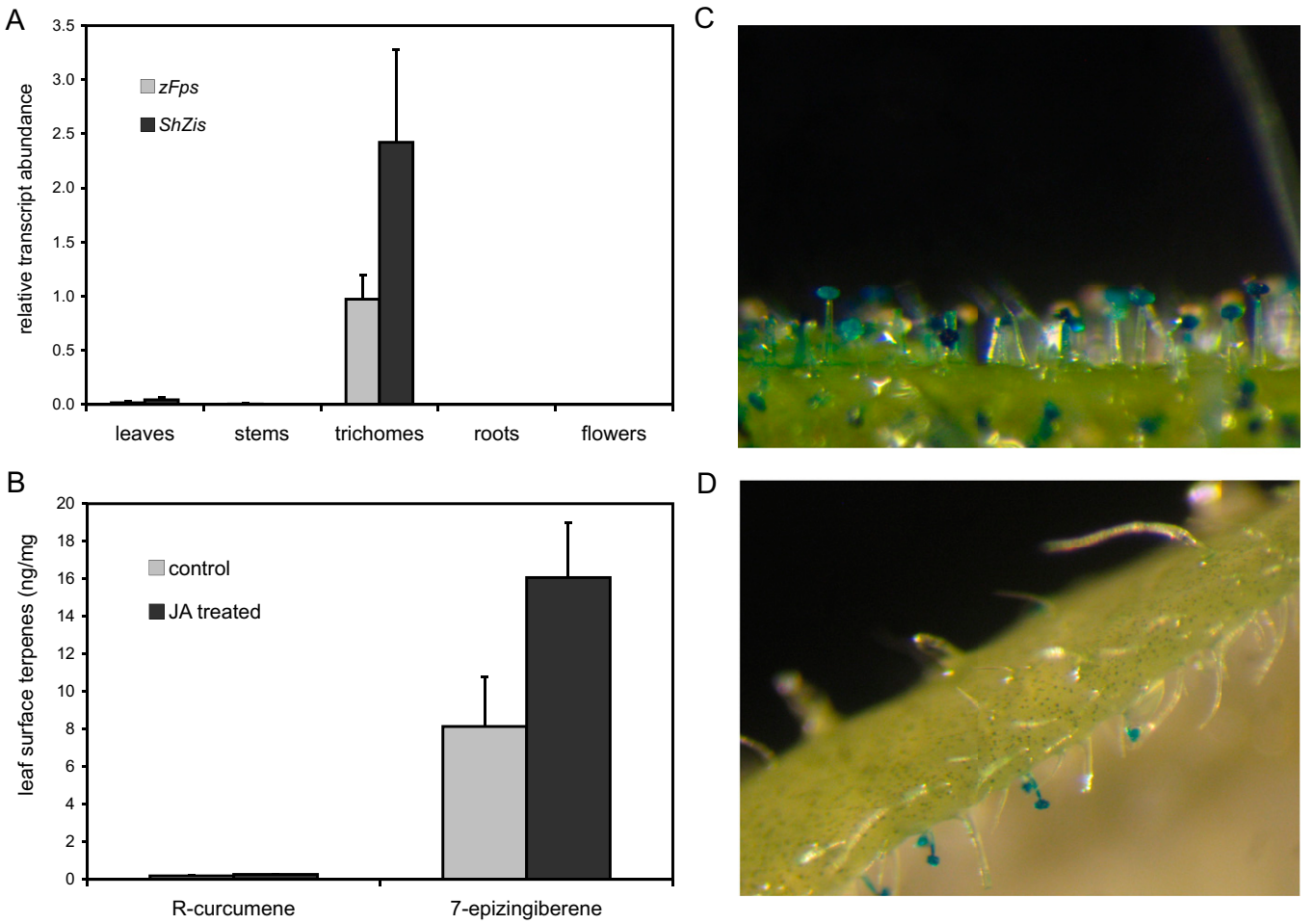

Fig. S4. 7-Epizingiberene production is trichome specific. (*A*) Tissue specific relative expression of *zFPS* (gray bars) and *ShZIS* (black bars) in *S. habrochaites* PI127826 determined by Q-RT-PCR. Bars represent means ± SE (n = 3). (*B*) Concentration of *R*-curcumene and 7-epizingiberene (ng/mg FW leaf) measured in 5 min hexane leaf wash in untreated plants (gray bars) and plants 24 h after jasmonic acid treatment (black bars). Bars represent means ± SE (n = 5). (*C*, *D*) Trichome-specific GUS expression in *S. lycopersicum* cv Moneymaker transformed with constructs containing GUS driven by the Methyl Ketone Synthase (MKS1) promoter (*A*) or by the Monoterpene synthase (MTS1) promoter (*B*). GUS activity was determined by staining with X-Gluc (16h).

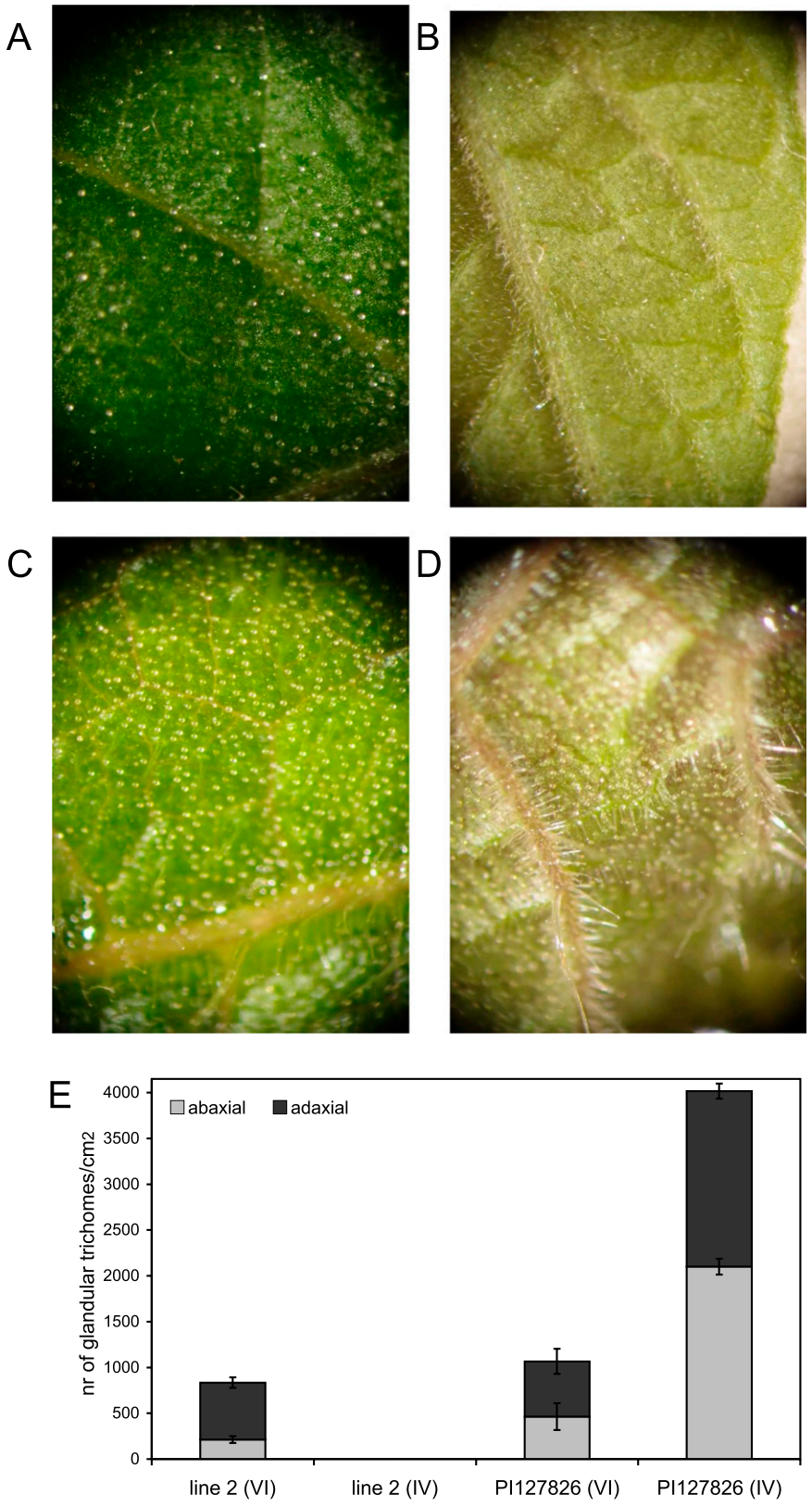

Fig. S5. Trichomes on adaxial (*A* and *C*) and abaxial (*B* and *D*) leaf surface of: *S. lycopersicum* cv Moneymaker transformed with pMKS1-zFPS and pMTS1-ShZIS (*A* and *B*) *S. habrochaites* PI1217826 (*C* and *D*). (*E*) number of glandular trichomes type VI and IV on abaxial (gray bars) and adaxial (black bars) side of the leaf of Moneymaker, transgenic line 2 and *S. habrochaites* PI127826. Means ± SE (n = 5).

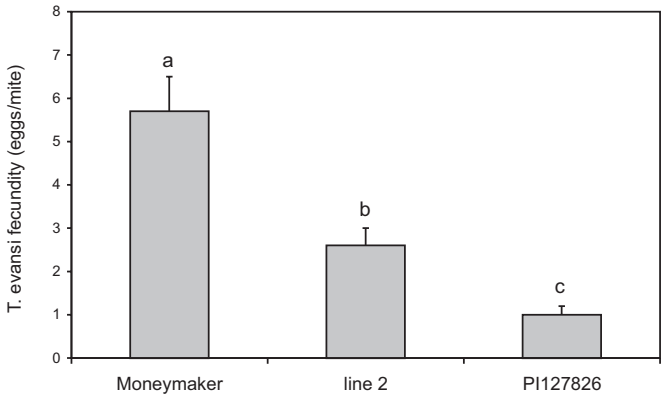

Fig. S6. *Tetranychus evansi* fecundity on *S. lycopersicum* cv Moneymaker, *S. lycopersicum* transgenic line 2 and *S. habrochaites* PI127826 displayed as number of eggs per mite per 4 d. Means ± SE (n = 60), different letters signify statistical differences ($P < 0.05$).

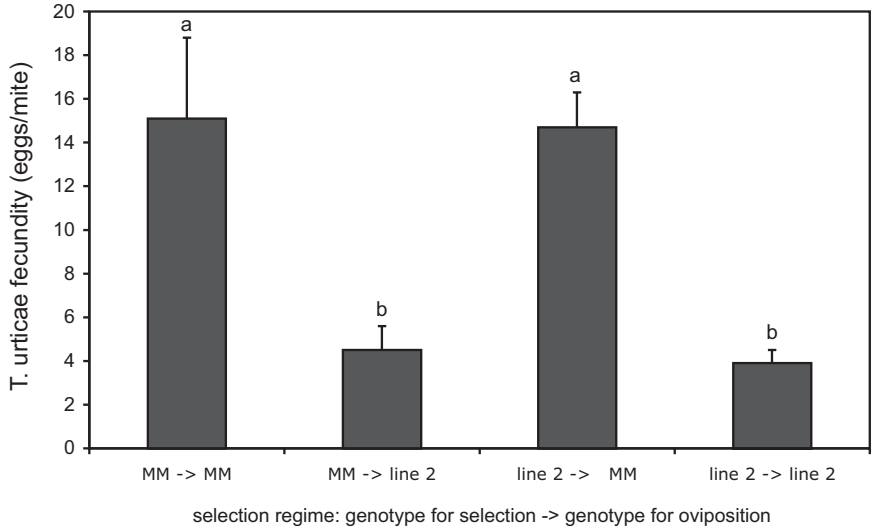

Fig. S7. Spider mite selection on 7-epizingiberene producing transgenic does not significantly improve performance. A total of 480 adult mites (*Tetranychus urticae)* were cultivated on *S. lycopersicum* cv Moneymaker (MM) or on *S. lycopersicum* transgenic line 2 for 5 wk (i.e., two full generation cycles) after which 200 adult mites of each were selected and allowed to produce eggs on fresh leaves (of the same genotype they had been selected on). After 48 h the adults were removed and their eggs allowed to develop into adults over a period of 14 d. These adult mites were used for the oviposition test depicted. Four combinations correspond to the four respective bars presented in the figure: (1) mites which had been selected on MM were allowed to produce eggs on MM leaf disks (MM -> MM with n = 60); (2) mites which had been selected on MM were allowed to produce eggs on Line 2 leaf disks (MM -> Line 2 with n = 60); (3) mites which had been selected on Line 2 were allowed to produce eggs on MM leaf disks (Line 2 -> MM with n = 60) and finally (4) mites which had been selected on Line 2 were allowed to produce eggs on Line 2 leaf disks (Line 2 -> Line 2 with n = 60). Bars represent mean number of eggs per mite per 4 d ± SE, different letters signify statistical differences ($P < 0.05$).

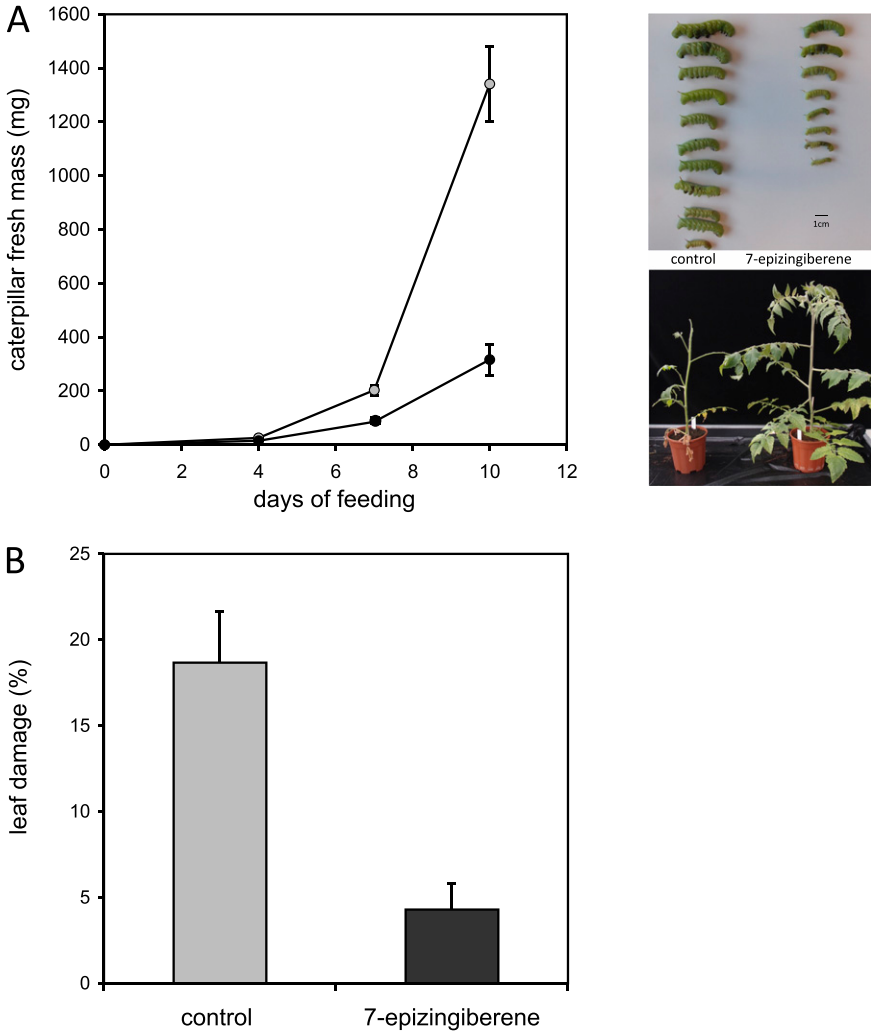

Fig. S8. (*A*) Effect of 7-epizingiberene in F2 tomato lines of an interspecific cross between *S. lycopersicum* cv Moneymaker and *S. habrochaites* PI127826 on the performance of the larvae of caterpillars (*Manduca sexta,* tobacco hornworm). Gray symbols: control plant (F2-200/2). Black symbols: F2 containing 7-epizingiberene (F2-100/21). Weight in mg, mean ± SE, $n = 16$ at the start of the assay, $P < 0.001$. (*Right*) Photos are *M. sexta* caterpillars and corresponding control and 7-epizingiberene producing F2 plants on which they fed ($t = 10$ d). (*B*) Effect of 7-epizingiberene in F2 tomato lines of an interspecific cross between *S. lycopersicum* cv Moneymaker and *S. habrochaites* PI127826 on the performance of neonates of *Leptinotarsa decemlineata* (Colorado Potato Beetle) in a choice assay between control plant (F2-200/2, gray bar) and a plant containing 7-epizingiberene (F2-100/21, black bar). Bars represent damage (%) to a leaf disk 48 h after start of the experiment, mean ± SE, $n = 15$, $P < 0.01$.

Table S1. Single nucleotide polymorphisms (SNPs) in ShZIS in five different *S. habrochaites* varieties all producing 7-epizingiberene

	SNP1	SNP2	SNP3	SNP4	SNP5	SNP6	SNP7	SNP8	SNP9	SNP10
Position (nt)	89	329	345	660	717	971	990	1011	1936	2321
Lines										
PI127826	G	G	T	A	T	C	A	A	A	C
LA0094	A	C	C	G	T	T	G/A	G/A	C	C
LA1978	A	C	C	A	C	T	G/A	G/A	C	T
LA2167	A	C	C	A	T	T	G/A	G/A	C	C
LA2650	A	C	C	G	T	T	G/A	G/A	C	T
Amino acid change	Arg (G) - Gln (A)	Ser (G) - Thr (C)	no	Met (G) - Ile (A)	no	Ala (C) - Val (T)	Met (G) - Ile (A)	no	Ile (A) - Leu (C)	Pro (C) - Leu (T)

no, no amino acid change.