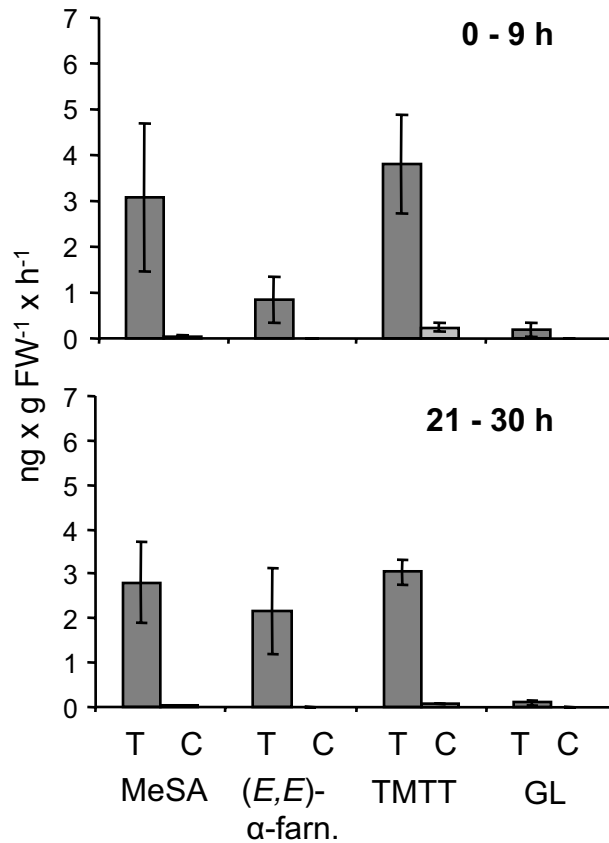
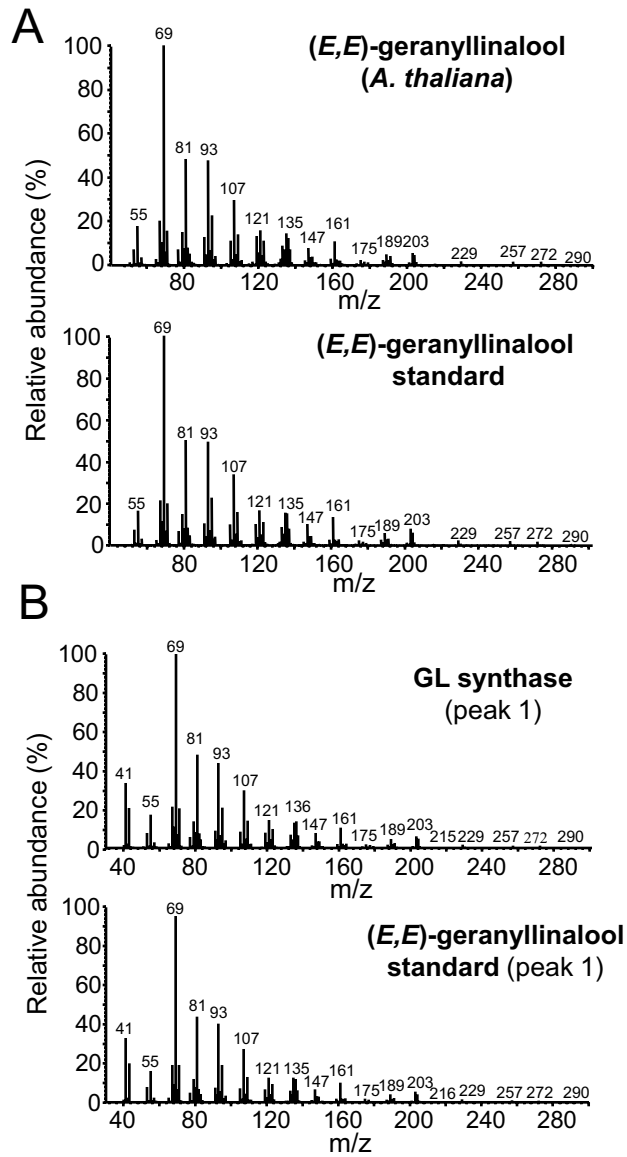


Supplemental Data. Herde et al. (2008). Identification and Regulation of TPS04/GES, an *Arabidopsis* Geranylinalool Synthase Catalyzing the First Step in the Formation of the Insect-Induced Volatile C₁₆-homoterpene TMTT.



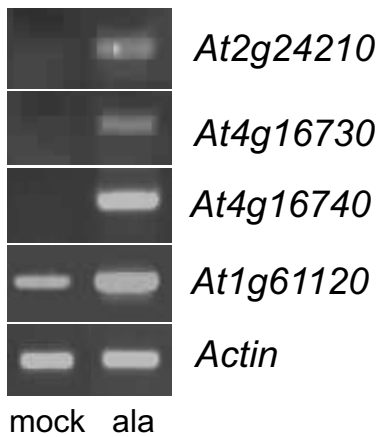
Supplemental Figure 1. Time course of volatile emissions from intact *Arabidopsis* rosette leaves in response to treatment with alamethicin.

Emission of the four major volatiles methyl salicylate (MeSA), (*E,E*)- α -farnesene, 4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT), and (*E,E*)-geranylinalool (GL) during two time periods of alamethicin-treatment (0-9 h, light period 1 and 21-30 h, light period 2) are shown. Volatiles were collected from detached rosette leaves by a closed-loop stripping procedure. The results represent the means \pm s.e of 3 replicates. T, alamethicin-treated; C, mock (0.1% EtOH).



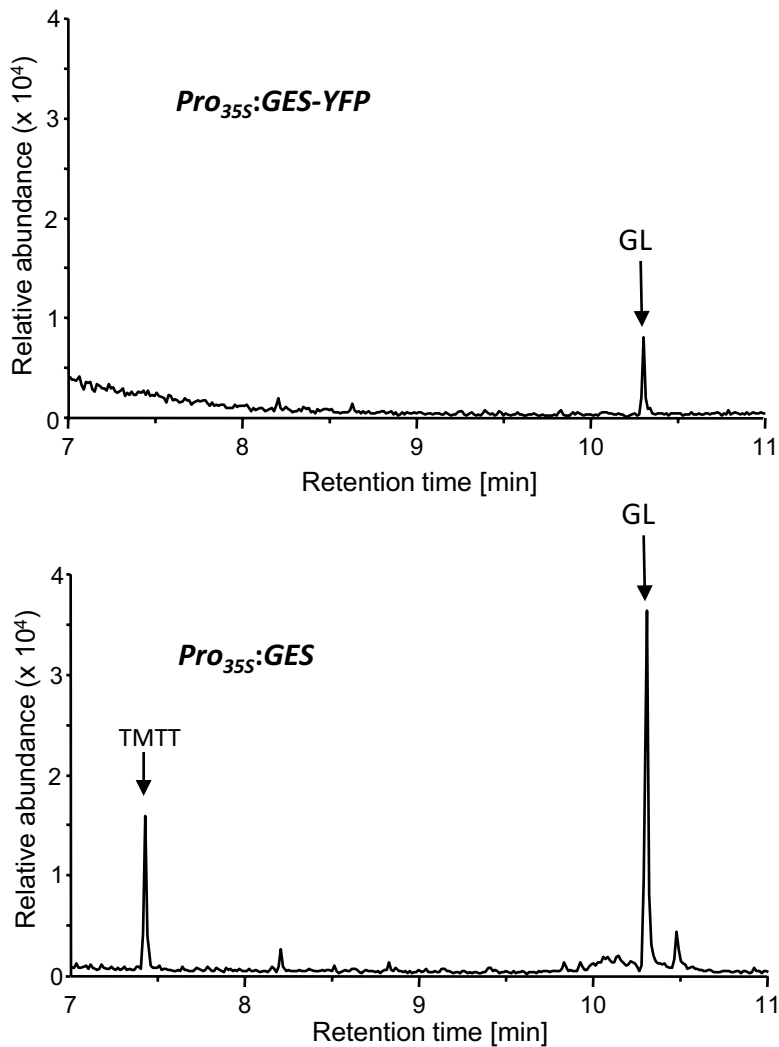
Supplemental Figure 2. Mass spectral analysis of (*E,E*)-geranyllinalool emitted from *Arabidopsis* leaves and synthesized by the recombinant GL synthase enzyme.

(A) The mass spectra of elicitor-induced (*E,E*)-geranyllinalool and of an authentic standard of (*E,E*)-geranyllinalool are shown. **(B)** The mass spectrum of enzyme-produced (*E,E*)-geranyllinalool is shown in comparison to that of an authentic (*E,E*)-geranyllinalool standard (peak 1 in Figure 4). Details of GC-MS analysis are described under Methods.

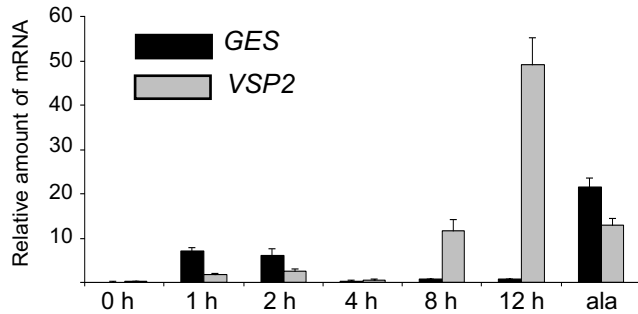


Supplemental Figure 3. Induction of *TPS* gene transcripts in *Arabidopsis* rosette leaves upon treatment with alamethicin.

Semi-quantitative reverse-transcriptase (RT) PCR analysis of transcript levels of the four *TPS* genes *At2g24210*, *At4g16730*, *At4g16740*, and *At1g61120* in detached rosette leaves after 30 h of treatment with alamethicin (ala) or ethanol (mock). Reactions with primers for *Actin8* were performed to judge equality of the cDNA template concentration. Some *At1g61120* transcript was detectable after 30 h of mock treatment which is consistent with the Northern blot analysis shown in Figure 3B.



Supplemental Figure 4. GC-MS analysis of volatiles emitted from leaves of *Pro*_{35S}:*GES-YFP* and *Pro*_{35S}:*GES* transformants. Thirty four-week-old plants grown on soil under long day conditions were used for continuous volatile collection for 31 h. GL: (E,E)-geranylinalool; TMTT: 4,8,12-trimethyltrideca-1,3,7,11-tetraene Chromatograms selected for 69 m/z are shown. The absence of TMTT in the *Pro*_{35S}:*GES-YFP* line might be due to lower GL and TMTT biosynthesis in this line as compared to *Pro*_{35S}:*GES* transformants. Volatiles were collected on charcoal traps and compounds were eluted with 40 μ L CH₂Cl₂. Samples were analyzed on an Agilent 6890 gas chromatograph coupled to an Agilent 5973 mass detector. Separation was performed on a CP8822 VF-23ms column (Varian) of 30 m x 0.25 mm i.d. x 0.25 μ m film-thickness. Helium was the carrier gas (flow rate 1 mL min⁻¹), a splitless injection (injection volume 2 μ L) was used, and a temperature gradient of 20°C min⁻¹ from 40°C (1 min hold) to 250°C was applied.



Supplemental Figure 5. Biological replicate of results shown in Figure 10D: Wound-induced gene expression of *Arabidopsis* *GES* as determined by real-time RT PCR.

Six-week-old hydroponically grown plants were wounded with forceps. Leaf material within ~3 mm adjacent to the wound marks was harvested. Material from 5 leaves was combined before extraction. Relative levels of *GES* and *VSP2* were determined by real-time RT PCR using SYBR Green I chemistry. Values were normalized to the expression of *At1g13320* (Protein Phosphatase Type 2). As a control, plants were treated with 5 $\mu\text{g}/\text{mL}$ alamethicin through the roots and RNA was harvested after 24 h. The results represent the means + s.e. of 3 technical replicates.

Supplemental Table 1. Statistical values for one-way ANOVA performed for data analysis shown in Figure 7E

	F	p
WT MeSA	25.278	<0.001
WT (E,E)- α -farnesene	0.815	0.461
WT TMTT	13.775	<0.001
WT (E,E)	nd	nd
<i>salk_039864</i> MeSA	31.329	<0.001
<i>salk_039864</i> (E,E)- α -farnesene	0.897	0.445
<i>salk_039864</i> TMTT	nd	nd
<i>salk_039864</i> GL	nd	nd
<i>Pro35S:GES #3</i> MeSA	27.475	<0.001
<i>Pro35S:GES #3</i> (E,E)- α -farnesene	8.010	0.004
<i>Pro35S:GES #3</i> TMTT	0.211	0.812
<i>Pro35S:GES #3</i> GL	0.929	0.417

Supplemental Table 2. Statistical values for one-way ANOVA performed for data analysis shown in Figure 9B

	F	p
MeSA	8.283	0.014
(E,E)- α -farnesene	2.570	0.119
TMTT	1.629	0.251
GL	0.936	0.462

Supplemental Table 3. List of Primers

Sequences are shown 5' to 3'. Restriction sites are shown in italics. The two sequences of primers used for recombinant PCR are distinguished by underline.

Name Sequence

P I	ATGGTAGG <i>TCTCAGCGCATGAAGTCTTCTTACGGTTCCTCC</i>
P II	ATGGTAGG <i>TCCATATCAGTAGAAGCATGGTGCGAATATCG</i>
P 1	TAGGGCCCGTCATAACGTGACTCCCTTAATTCTCATGTATG
P 2	TGGGCCCGCTAGCGATGTGCTGCAAGGCGATTAAGTTGGGTAACGC
P 3	CTATATAAGGAAGTTCATTTTCATTTGGAGAGGAA <u>CACTCAATCTAAAGACCATCCTCTAGACAGAA</u> <u>AAC</u>
P 4	TTTCGCCGTCTT <i>CGTCTG</i> CCATCAGCCACCGTTG
P 5	AGCTTCCTAGGAGGCCTGAATTCCTGCAGGA
P 6	AGCTTCCTGCAGGGAATTCAGGCCTCCTAGGA
P 7	AGAATCATCCAGGTCTGGAGATTGAGGACTC
P 8	TGGTAAATCAGTGGCGATACTGATGGAAGAG
P 9	GCAATCGATATTCGCACCATGCTTCTACTCCGGGGCGATCGCTTAATATCTTATCATTATCTATAT TATATATG
P 10	CCCGGGGCGGAGGGATGGTGAGCAAGGGCGA
P 11	GCGATCGCTTACTTGTACAGCTCGTCCA
P 12	GGGACACACATCGTCAATG
P 13	GCTGCGACGCAACAATC'
P 14	GGGGACAAGTTTGTACAAAAAAGCAGGCTATCGATCAATGATGATCAATTAGTCACTTCAGAGAG
P 15	GGGGACCACTTTGTACAAGAAAGCTGGGTAAAATATGGGTCACTAATCTGCGTGTGTTTTATAG
P 16	GATAGCGAACCAACGAGGAT
P 17	CTTGTGTTGTAGCACTTCAGAAA
P 18	CAAACATAACAATAAACCATACCATAA
P 19	GCCAAGAGCAAGAGAAGTGA
P 20	GCACAGCAATCGGGTATAAAG
P 21	AAGCAGCGTAATCGGTAGG
P 22	CCCAACACCAACAAAACG
P 23	CATGCATTGAGACATGCG
P 24	CTCCGTCCTATCCGACCAC
P 25	CTGGGCTTCCGTGGC
P 26	AACCTTAGACATTCTTGGCTC
P 27	GGGTGGACGAGCAAGGAC
P 28	GGGACACACATCGTCAATG
P 29	GCTGCGACGCAACAATC
P 30	ATGAAGATTAAGGTCGTGGCAC
P 31	GTTTTTATCCGAGTTTGAAGAGGC