

Current Biology

Tissue-Specific Emission of (*E*)- α -Bergamotene Helps Resolve the Dilemma When Pollinators Are Also Herbivores

Highlights

- In wild tobacco, both flowers and herbivory-induced leaves emit (*E*)- α -bergamotene
- Floral (*E*)- α -bergamotene increases *Manduca sexta* moth-mediated pollination success
- Herbivory-induced (*E*)- α -bergamotene mediates indirect defense against *M. sexta* larvae
- Expression of *NaTPS38* regulates both floral and herbivory-induced (*E*)- α -bergamotene

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In Brief

Plants face a dilemma when their pollinators are also herbivores. Zhou et al. find that wild tobacco resolves this dilemma through tissue-specific emission of a sesquiterpene, regulated by *NaTPS38*, which increases pollination success in flowers while defending against herbivores in leaves.



Tissue-Specific Emission of (*E*)- α -Bergamotene Helps Resolve the Dilemma When Pollinators Are Also Herbivores

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SUMMARY

More than 87% of flowering plant species are animal-pollinated [1] and produce floral scents and other signals to attract pollinators. These floral cues may however also attract antagonistic visitors, including herbivores [2]. The dilemma is exacerbated when adult insects pollinate the same plant that their larvae consume. It remains largely unclear how plants maximize their fitness under these circumstances. Here we show that in the night-flowering wild tobacco *Nicotiana attenuata*, the emission of a sesquiterpene, (*E*)- α -bergamotene, in flowers increases adult *Manduca sexta* moth-mediated pollination success, while the same compound in leaves is known to mediate indirect defense against *M. sexta* larvae [3, 4]. Forward and reverse genetic analyses demonstrated that both herbivory-induced and floral (*E*)- α -bergamotene are regulated by the expression of a monoterpene-synthase-derived sesquiterpene synthase (*NaTPS38*). The expression pattern of *NaTPS38* also accounts for variation in (*E*)- α -bergamotene emission among natural accessions. These results highlight that differential expression of a single gene that results in tissue-specific emission of one compound contributes to resolving the dilemma for plants when their pollinators are also herbivores. Furthermore, this study provides genetic evidence that pollinators and herbivores interactively shape the evolution of floral signals and plant defense.

RESULTS AND DISCUSSION

Nicotiana attenuata Flowers and Herbivory-Induced Leaves Emit (*E*)- α -Bergamotene in the Night and Daytime, Respectively

A previous study showed that (*E*)- α -bergamotene, which is emitted from local and systemic leaves of herbivory-induced plants and mediates indirect defense [3, 4] by attracting predatory *Geocoris* spp., is also emitted from floral tissues [5]. We analyzed the kinetics of both herbivory-induced and floral (*E*)- α -bergamotene emission in *N. attenuata*. Flowers of *N. attenuata* mainly emit (*E*)- α -bergamotene at night. Their (*E*)- α -bergamotene emission levels increased rapidly immediately after corolla opening at 6 p.m. and reached the highest emission levels between 8 p.m. and 1 a.m., after which levels decreased during the subsequent morning (Figure 1). This kinetic of floral (*E*)- α -bergamotene emission was similar to another major floral attractant, benzyl acetone (BA), whose emission is coordinated with the activity of the *M. sexta* moth [6].

In leaves, constitutive (*E*)- α -bergamotene emission peaked in the morning and subsequently decreased until 2 a.m., but overall emission levels were very low. After simulated herbivory by wounding leaves with pattern wheels and adding *M. sexta* oral secretions (OSs) to the wounds, we found that the emission of (*E*)- α -bergamotene had two peaks during the daytime. The first peak was in the afternoon (12 p.m.–8 p.m.) on the day of elicitation, and the second one was during the following morning (6 a.m.–10 a.m.). Additional experiments also showed that the day emission pattern of herbivory-induced (*E*)- α -bergamotene was largely independent of the elicitation time (Y. Joo, M.C.S, J.K. Goldberg, S.-G. Kim, F.Y., C. Brütting, and I.T.B., unpublished data). Furthermore, the timing of herbivory-induced (*E*)- α -bergamotene emission matched the activity and

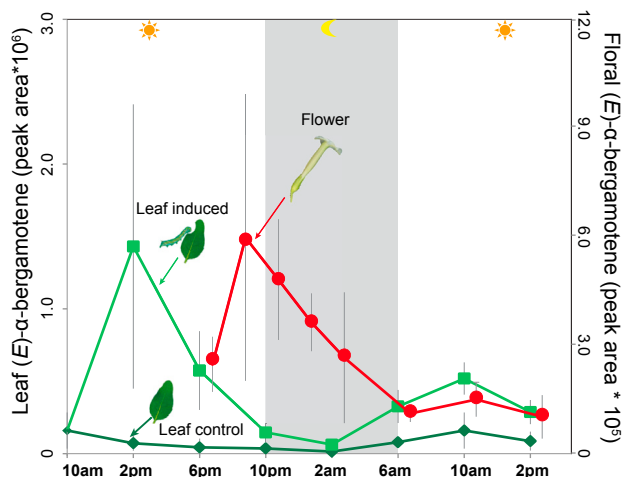


Figure 1. *Nicotiana attenuata* Emits (*E*)- α -Bergamotene in Both Flowers and Herbivory-Induced Leaves

Emission kinetics of (*E*)- α -bergamotene in the leaf (green) and the flower (red) are shown. The x axis indicates the sampling time, and the y axis denotes the abundance of (*E*)- α -bergamotene level in leaves (left) and flowers (right). Dark-green- and light-green-colored lines refer to control (unwounded and uninduced) and OS-induced (wounded with *M. sexta* oral secretion added) leaf samples, respectively. The elicitations were carried out at 8 a.m. The leaf volatiles were collected for 4 hr, and the middle time points are shown. The floral volatiles were collected for 1 hr, and the middle time points are shown. Data are presented as mean \pm SD.

abundance of *Geocoris* spp. predators in the field (Y. Joo, M.C.S, J.K. Goldberg, S.-G. Kim, F.Y., C. Brütting, and I.T.B., unpublished data).

The Terpene Synthase *NaTPS38* Is Involved in the Emission of Both Herbivory-Induced and Floral (*E*)- α -Bergamotene

To examine whether herbivory-induced and floral (*E*)- α -bergamotene share the same biosynthetic machinery, we correlated herbivory-induced and floral (*E*)- α -bergamotene emissions among 23 natural accessions of *N. attenuata*. We reasoned that, if the same molecular machinery controls both herbivory-induced and floral (*E*)- α -bergamotene, the emission levels from these two tissues should co-vary among different genotypes. Indeed, the results showed that the levels of (*E*)- α -bergamotene in flowers and herbivory-induced leaves are highly variable and significantly correlated among 23 genotypes ($p = 3.2E-6$; $R^2 = 0.65$; Figure 2A), indicating that natural variations of (*E*)- α -bergamotene in flowers and herbivory-induced leaves share the same genetic basis.

To identify the genetic basis underlying the variation in (*E*)- α -bergamotene emission, we performed quantitative trait loci (QTL) mapping using an *N. attenuata* advance intercross recombinant inbred line (AI-RIL) population developed by crossing two inbred lines originating in Arizona (AZ) and Utah (UT), USA (Supplemental Information), which differ in both herbivory-induced and floral (*E*)- α -bergamotene emissions (Figure 2B). We measured the herbivory-induced (*E*)- α -bergamotene emission among 256 individuals of the AI-RIL population (Supplemental Information). The analysis showed that, within a 95% confidence interval, the genetic control of

herbivory-induced (*E*)- α -bergamotene was mapped to two QTL loci on linkage groups 1 and 2 (Figure 2C, QTL1 and QTL2). By fitting a multiple QTL model, QTL1 and QTL2 showed significant dominant and additive effects, respectively (Table S1).

We searched candidate genes in the corresponding genomic information of the two candidate loci in *N. attenuata* and their syntenic genomic region in tomato. The major QTL locus (QTL2) overlapped with a terpene synthase (TPS) cluster that contains members of the TPS-b subclade. In tomato, this cluster includes *SITPS25*, *SITPS26* (pseudogene), *SITPS27*, and *SITPS38* [7]. Among these, whereas *SITPS25* and *SITPS27* are highly similar to the grape *VvTPS47* that produces either (*E*)- β -ocimene using geranyl pyrophosphate (GPP) or (*E*)- α -farnesene using farnesyl pyrophosphate (FPP), *SITPS38* produces (*E*)- α -bergamotene using FPP in vitro [7]. Searching the available genomic and transcriptomic information of *N. attenuata* (both UT and AZ genotypes), we identified *NaTPS38* as the ortholog of *SITPS38* and *NaTPS25*—which was not found in UT but was found in the AZ genome—as the ortholog of *SITPS25/27*.

Examining the expression profile of herbivory-induced transcriptomic responses in *N. attenuata* (UT genotype) showed that *NaTPS38* is highly induced after herbivory in leaves (Figure 3A). Among different floral tissues, *NaTPS38* was highly expressed in the corolla tube, but not in the floral limb (Figure 3B), consistent with the previously reported tissue-specific emission of (*E*)- α -bergamotene in flowers [5].

To measure the TPS activity of *NaTPS38* and *NaTPS25* in vitro, we heterologously expressed these two genes in *Escherichia coli*. The recombinant *NaTPS25* converted the substrate GPP into (*E*)- β -ocimene as the only product (data not shown); *NaTPS38* converted the substrate (*E,E*)-FPP into (*E*)- α -bergamotene as the sole product (Figures 3C and 3D). Additional tests with (*E,E*)-FPP revealed that *NaTPS25* does not convert (*E,E*)-FPP into sesquiterpenes. These results indicate that *NaTPS38* is likely the gene involved in (*E*)- α -bergamotene biosynthesis.

To determine the function of *NaTPS38* in vivo, we silenced the expression of this gene in *N. attenuata* UT using virus-induced gene silencing (VIGS) (Supplemental Information). Because the emission of herbivory-induced (*E*)- α -bergamotene is mediated by jasmonate signaling [10], we measured both constitutive and methyl jasmonate (MeJA)-induced (*E*)- α -bergamotene emission in leaves. Silencing *NaTPS38* reduced the emission of (*E*)- α -bergamotene in flowers and in uninduced and MeJA-induced leaves (Figures 3E–3H).

We further measured the transcript abundance of *NaTPS38* in both flowers and herbivory-induced leaves among the 23 natural accessions using qPCR. *NaTPS38* transcripts were quantifiable in all 23 accessions, but relative transcript abundance varied. *NaTPS38* transcript abundance in flowers and herbivory-induced leaves were significantly correlated with the emission of (*E*)- α -bergamotene in these two tissues among the 23 accessions (Figure S1; flowers: $p = 0.0098$, $R^2 = 0.28$; herbivory-induced leaves: $p = 1.0E-8$, $R^2 = 0.77$). These results showed that the emissions of both herbivory-induced and floral (*E*)- α -bergamotene are mediated by *NaTPS38* in *N. attenuata*.

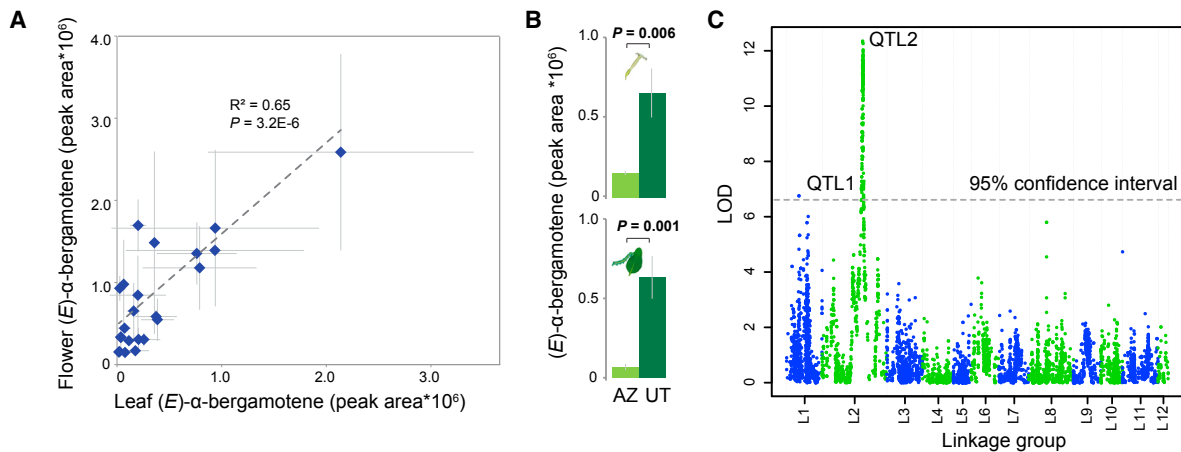


Figure 2. Genetic Basis of (*E*)- α -Bergamotene Emission in *N. attenuata*

(A) Floral (y axis) and herbivory-induced leaf (x axis) (*E*)- α -bergamotene emissions are correlated among natural accessions. Data are shown as mean \pm SD. (B) Both floral (top panel) and herbivory-induced (bottom panel) (*E*)- α -bergamotene emissions are significantly different between AZ and UT genotypes. Data are shown as mean \pm SEM. For both (A) and (B), the measurement of herbivory-induced and floral emission (*E*)- α -bergamotene was performed for the periods of 9 p.m.–10 p.m. and 12 p.m.–4 p.m., respectively. (C) Herbivory-induced (*E*)- α -bergamotene is mapped to two QTL loci. Two QTL loci on linkage group 1 (QTL1) and 2 (QTL2) are marked. The 95% confidence interval is indicated with a dashed line. See also [Table S1](#).

***NaTPS38* Evolved from a Monoterpene Synthase and Was Likely under Positive Selection**

Whereas the product of *NaTPS38* is a sesquiterpene, *NaTPS38* is located in the monoterpene synthase gene cluster (TPS-b clade). To understand the molecular evolution of *NaTPS38*, we constructed a phylogenetic tree using *NaTPS38* and members of TPS-b clade from *N. attenuata*, tomato, grape, and *Arabidopsis thaliana*. The results showed that *TPS38* can be found in both *Nicotiana* and tomato but is absent in grape and *A. thaliana*, suggesting that *TPS38* is Solanaceae-specific ([Figure S2A](#)). Indeed, we found a single copy of *TPS38* in available genomic datasets from all diploid solanaceous species, including *Petunia axillaris* and *N. obtusifolia* ([Table S2](#)).

Whereas monoterpene synthases are often localized in plastids and contain chloroplast transit peptides [11], sesquiterpenes are predominantly synthesized in the cytosol. To investigate the localization of *TPS38* in *N. attenuata*, we measured the subcellular localization of *NaTPS38* fused to yellow fluorescent protein (YFP) by transient expression assays in leaves of *N. attenuata*. The fusion protein of YFP-*NaTPS38* was localized to the cytosol ([Figure S3](#)), suggesting that *TPS38* lost its chloroplast transit peptide (cTP) from its ancestor.

In addition to changes in subcellular localization, *in vitro* assays also showed that both tomato and *Nicotiana* *TPS38* specifically use (*E,E*)-FPP as a substrate, which differed from their ancestors, which use GPP as their primary substrate [7]. This indicated that amino acid changes resulting in an altered substrate specificity might have occurred before the speciation event between *Nicotiana* and *Solanum*. To examine whether this change in substrate utilization was under positive selection, we performed a maximum-likelihood-based analysis of synonymous mutations (d_S ; preserving the amino acid sequence) versus nonsynonymous mutations

(d_N ; altering the amino acid sequence). We found that the ratio of d_N/d_S on the *TPS38* clade before the split of *Solanum* and *Nicotiana* was significantly higher than that of other branches ($p = 9.8E-14$), indicating that the evolution of the *TPS38* branch was under either positive or relaxed purifying selection.

To further understand the evolution of *TPS38* at a population level, we analyzed the whole-genome re-sequencing data from 23 natural accessions of *N. attenuata*. Based on sliding-window analyses, we found a sharp decrease of nucleotide diversity at the genomic location of *NaTPS38*, as measured by Tajima's *D*, Fu, and Li's *F* and site frequency spectrum statistics ([Figures S2B–S2D](#)). This suggests that *NaTPS38* was likely under positive selection in *N. attenuata* populations.

Floral (*E*)- α -Bergamotene Increases Pollination Success in *N. attenuata*

Because the expression of *NaTPS38* is associated with both herbivory-induced and floral (*E*)- α -bergamotene, the evolution of this gene is likely shaped by the ecological functions of (*E*)- α -bergamotene in both flowers and herbivory-induced leaves. Whereas herbivory-induced (*E*)- α -bergamotene is known to improve the fitness of *N. attenuata* by attracting predators of *M. sexta* larvae, thereby acting as an indirect defense [3, 4], the ecological functions of floral (*E*)- α -bergamotene remained largely unknown.

The night-emission pattern of floral (*E*)- α -bergamotene—which is similar to that of benzyl acetone (BA), the major attractant of *M. sexta* moths as pollinators in *N. attenuata* [6, 12]—suggests that floral (*E*)- α -bergamotene might be involved in modifying *M. sexta* moth pollination behavior. Tests based on electroantennograms showed weak responses of *M. sexta* antennae to (*E*)- α -bergamotene at the concentration present in the floral headspace ([Table S3](#)), which was consistent with its

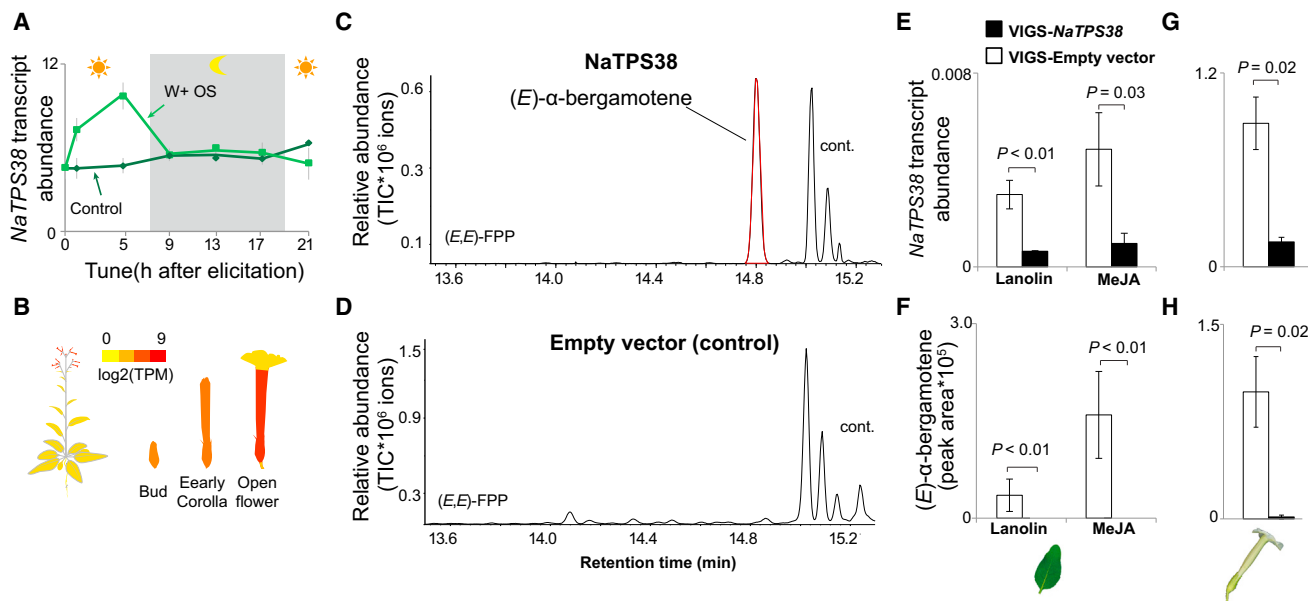


Figure 3. *NaTPS38* Is Required for Both Herbivory-Induced and Floral (*E*)- α -Bergamotene Emission in *N. attenuata*

(A) The expression kinetics of *NaTPS38* in leaves. Dark-green- and light-green-colored lines refer to control (undamaged) and OS-induced leaves, respectively. The y axis shows the relative expression level measured by microarray. Data are shown as mean \pm SEM. The data were extracted from the microarray dataset in Kim et al. [8].

(B) A heatmap depicts tissue-specific expression of *NaTPS38* in *N. attenuata*. The relative expression levels of *NaTPS38* among different tissues were extracted from the *N. attenuata* data hub [9]. The color gradient represents the relative expression value ($\log_2(\text{TPM})$). TPM, transcripts per million.

(C and D) Heterologously expressed *NaTPS38* produces (*E*)- α -bergamotene as the sole product in vitro (C). The empty vector control is shown in (D). Gas chromatography (GC) chromatograms of enzyme products from recombinant *NaTPS38* expressed in *E. coli* and incubated with the substrate (*E,E*)-FPP are depicted. Cont. refers to contaminations. The peak of the target product is marked in red.

(E–H) Silencing the expression of *NaTPS38* using VIGS in flowers and leaves. For leaves, both constitutive (control, with lanolin) and MeJA (lanolin + MeJA)-induced levels of *NaTPS38* expression and (*E*)- α -bergamotene emissions were measured. p values were calculated using Student's t tests. Data are shown as mean \pm SEM.

(E and G) In comparison to the empty vector (EV) control, the relative transcript abundance of *NaTPS38* was reduced in both leaves (E) and flowers (G) of VIGS-*NaTPS38* plants.

(F and H) In comparison to EV plants, the levels of (*E*)- α -bergamotene emission in both leaves and flowers of VIGS-*NaTPS38* plants were significantly lower.

See also Figures S1–S3 and Table S2.

inability to attract moths in wind tunnel assays (data not shown). We hypothesized that (*E*)- α -bergamotene might act as a stimulus to the chemosensory sensilla at the tip of *M. sexta*'s proboscis, which also house chemosensory receptors detecting BA [13]. Repeating these recordings with purified (*E*)- α -bergamotene indeed revealed the neuronal responses of *M. sexta*'s proboscis at concentrations present in the floral headspace (Figure 3A, insert; Table S3). Therefore, we specifically examined the effects of (*E*)- α -bergamotene on proboscis-based attraction in a Y-maze setup [13].

We first examined the effect of the pure compound on the probing behavior of *M. sexta* moths. In comparison to the solvent, (*E*)- α -bergamotene significantly increased the probing time (Figure 4A; $p = 0.002$) of *M. sexta* moths, which is associated with increased pollination success [13]. To further examine the effects of (*E*)- α -bergamotene on the probing behavior of *M. sexta* moths in the natural floral volatile background, we tested the probing preference of *M. sexta* moths between the headspaces of (*E*)- α -bergamotene-emitting (VIGS-EV) and non-emitting (VIGS-*NaTPS38*) flowers. Probing time of *M. sexta* moths was significantly longer in (*E*)- α -bergamotene-emitting flowers

compared to the (*E*)- α -bergamotene-deficient flowers (Figures 4B and S4; $p = 0.016$; Supplemental Information). To test whether the observed probing time behavior changes have direct consequences on plant fitness, we further performed an experiment under semi-natural conditions in a large outdoor tent [6], offering individual *M. sexta* moths antherectomized flowers with and without biologically relevant amounts of (*E*)- α -bergamotene supplemented in the corolla tube (Supplemental Information). Supplementation of (*E*)- α -bergamotene significantly increased both the likelihood of capsule formation ($p = 0.05$; binomial test) and the total seed numbers per flower (Figure 4C; $p = 0.03$; Wilcoxon signed-rank test). Together, these results show that floral (*E*)- α -bergamotene increases *M. sexta* moth-mediated pollen transfer between plants and thus outcrossing rate in *N. attenuata*.

In conclusion, whereas *N. attenuata* flowers express *NaTPS38* and emit (*E*)- α -bergamotene in the night to facilitate pollination by *M. sexta* moths [3, 4], these same pollinators often lay eggs on the leaves of the same plant [6]; when the leaves are attacked by *M. sexta* larvae, they express the same gene and emit the same compound during the day to attract the predators

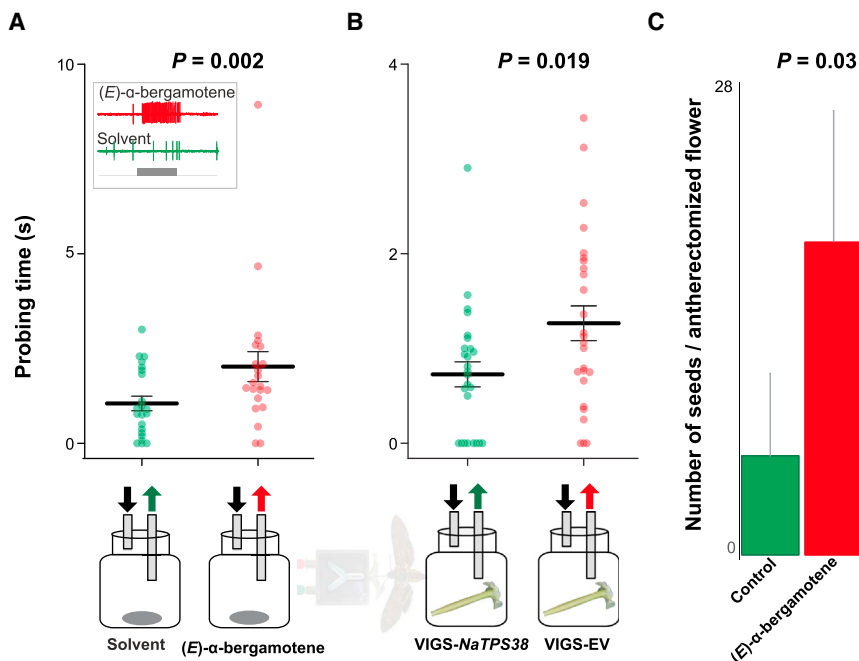


Figure 4. *N. attenuata* Floral (*E*)- α -Bergamotene Increases Probing Time and *M. sexta* Moth-Mediated Pollination Success

(A) *M. sexta* moths probed significantly longer in the Y-maze arm in which (*E*)- α -bergamotene was present than in the arm with solvent present ($p = 0.002$; Wilcoxon signed-rank test). Inset in (A) shows a representative response of a sensillum on the tip of *M. sexta*'s proboscis to (*E*)- α -bergamotene (up) and the solvent control (low). Black bar at the bottom indicates stimulus duration (0.5 s).

(B) *M. sexta* moth probed significantly longer in the Y-maze arm with the floral headspace from VIGS-empty vector (EV) flowers than in the one from VIGS-*NaTPS38* flowers ($p = 0.019$; Wilcoxon signed-rank test).

(C) (*E*)- α -bergamotene supplementation in corolla tubes increased *M. sexta* moth-mediated pollination success ($p = 0.03$; Wilcoxon signed-rank test). Data are shown as mean \pm SEM.

See also Figure S4 and Tables S3 and S4.

of *M. sexta* larvae, which subsequently reduce herbivore damage [3, 4, 14]. Thus, tissue- and time-specific expression of *NaTPS38* and its product (*E*)- α -bergamotene mediate both defense and pollinator attraction and contribute to resolving the dilemma that *N. attenuata* plants face because its preferred pollinator is also a voracious herbivore. Furthermore, these results suggest that interactions between herbivores and pollinators can act synergistically or additively in shaping plant evolution [2].

ACCESSION NUMBERS

The accession number for the GBS data of the AI-RIL population reported in this paper is NCBI: PRJNA378521.

SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures, four tables, and Supplemental Experimental Procedures and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2017.03.017>.

AUTHOR CONTRIBUTIONS

Conceptualization, S.X.; Methodology, W.Z., S.X., E.M., A.H., F.B., M.K., F.Y., R.L., M.C.S., B.S.H., and D.K.; Investigation, W.Z., S.X., A.K., E.M., A.H., H.G., F.B., N.L., T.G.K., J.B., and D.K.; Writing – Original Draft, S.X. and W.Z.; Writing – Review and Editing, S.X., W.Z., I.T.B., A.H., and M.C.S.; Funding Acquisition, S.X., I.T.B., and B.S.H.; Resources, S.X., B.S.H., I.T.B., and D.K.; Supervision, S.X.

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Supplemental Information

Tissue-Specific Emission of (*E*)- α -Bergamotene

Helps Resolve the Dilemma

When Pollinators Are Also Herbivores

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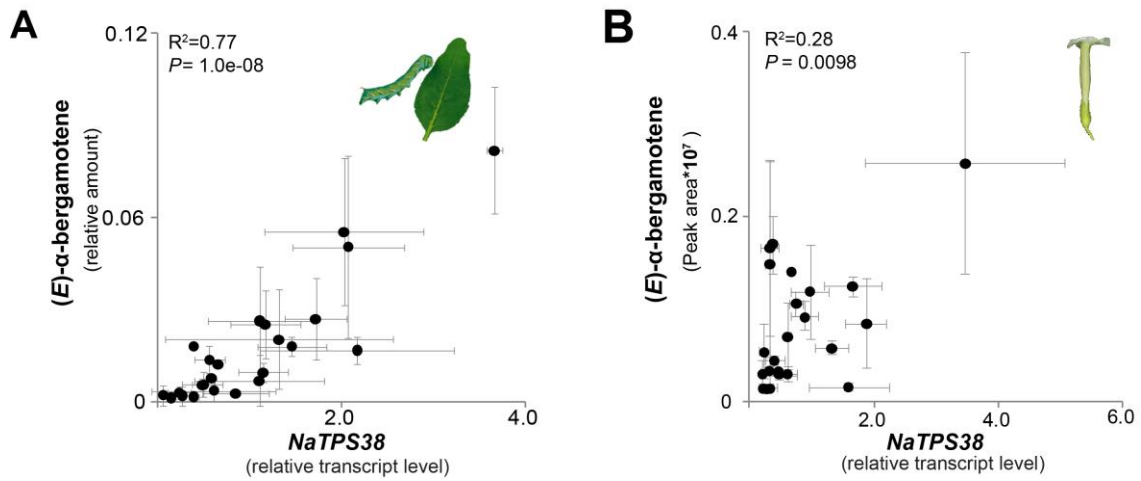


Figure S1. Among 23 natural accessions, relative transcript abundances of *NaTPS38* correlates with levels of (*E*)- α -bergamotene emission in both flowers (B) and herbivory-induced leaves (A), related to Figure 3.

The x-axis indicates the relative transcript abundance of *NaTPS38* measured at 4pm (leaves) or 10 pm (flowers); Y-axis refers to the relative amount of (*E*)- α -bergamotene measured between 12 and 4 pm (leaves) or between 9 and 10 pm (flowers). Data is shown as mean \pm SD.

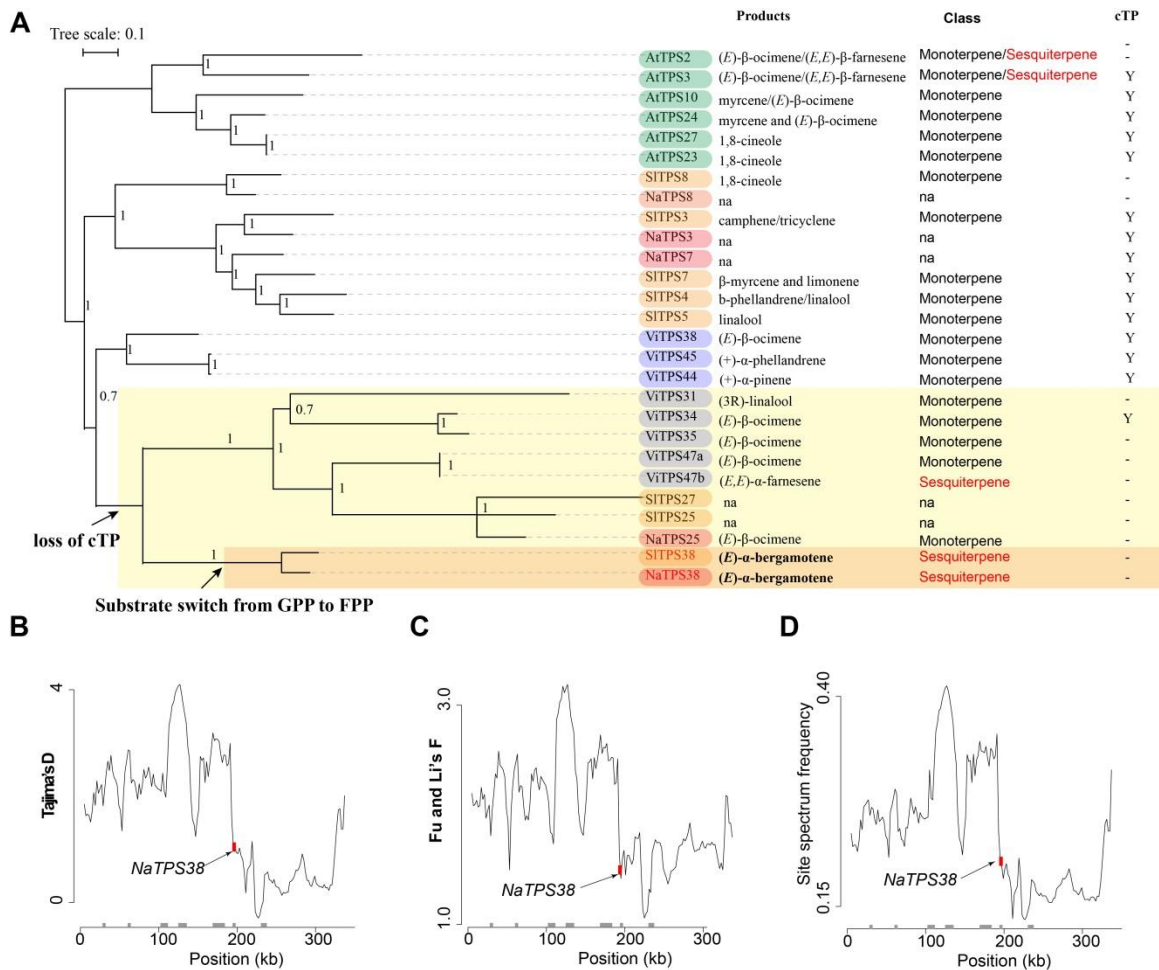


Figure S2. *NaTPS38* evolved from a monoterpene synthase and was under positive selection in *N. attenuata*, related to Figure 3.

A, a phylogenetic tree of genes from TPSb-clade in four plant species is shown. The number at each branch indicates an approximated *Bayesian* value. The predicted loss of chloroplast transit peptides (cTP) and substrate switching are shown on the tree. The leaf color of the phylogenetic tree refers to different species. Green: *Arabidopsis thaliana* (*At*); orange: *Solanum lycopersicum* (*Sl*); light blue: *Vitis vinifera* (*Vi*); pink: *Nicotiana attenuata* (*Na*). The products for each enzyme were summarized from previous publications and were classified as monoterpene or sesquiterpene. 'na' indicates that the product is unknown. The chloroplast transit peptides (cTP) for each TPS were predicted with Chloro P [S1]. 'Y' and '-' indicate predicted cTP presence and absence, respectively. **B-D**, a significant reduction of nucleotide diversity at the genomic region near *NaTPS38*. Sliding window plot of Tajima's D (**B**), Li and Fu's F (**C**) and site spectrum frequency (**D**) statistics are shown for the scaffold where *NaTPS38* is located. In each panel, the red bar depicts *NaTPS38*, and gray rectangles at the bottom depict locations of other protein coding genes. The genome-wide Tajima's D, Fu and Li's F and SFS are positive, which suggest either a population reduction in the past or the unique fire chasing behavior of *N. attenuata* resulting a subset of genetic diversity were collected from a large seed bank.

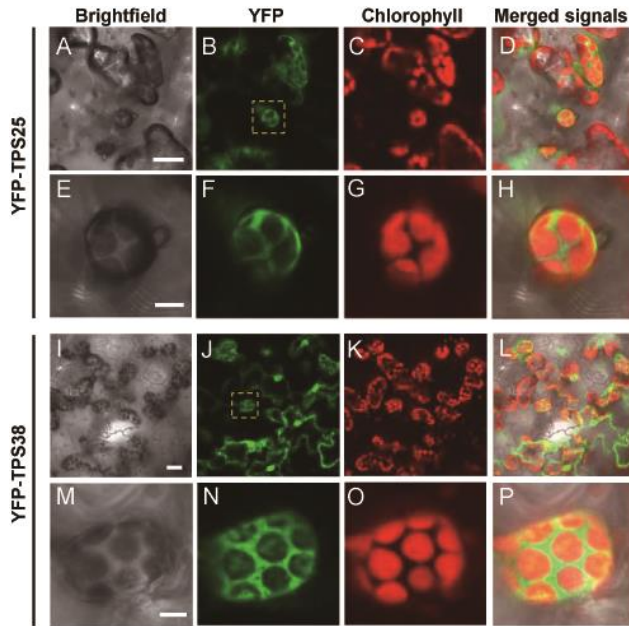


Figure S3. Subcellular localization of NaTPS25 and NaTPS38, related to Figure 3.

A-H, subcellular localization of YFP-TPS25; **I-P**, subcellular localization of YFP-TPS38. **E-H** and **M-P** are the zoomed view of **A-D** and **I-L**, respectively. The complete open reading frame of NaTPS25 (YFP-TPS25, A-H, T-V) and NaTPS38 (YFP-TPS38, I-P, W-Y) were fused downstream of YFP and transiently expressed in *N. attenuata* leaves. YFP fluorescence indicates the location of each fusion protein (green); the location of the chloroplasts was determined by chlorophyll autofluorescence (red). Merged signals provide a view of all fluorescent signals emitted from the leaf area. Scale bar in A, I: 20 μm ; in E, M: 5 μm .

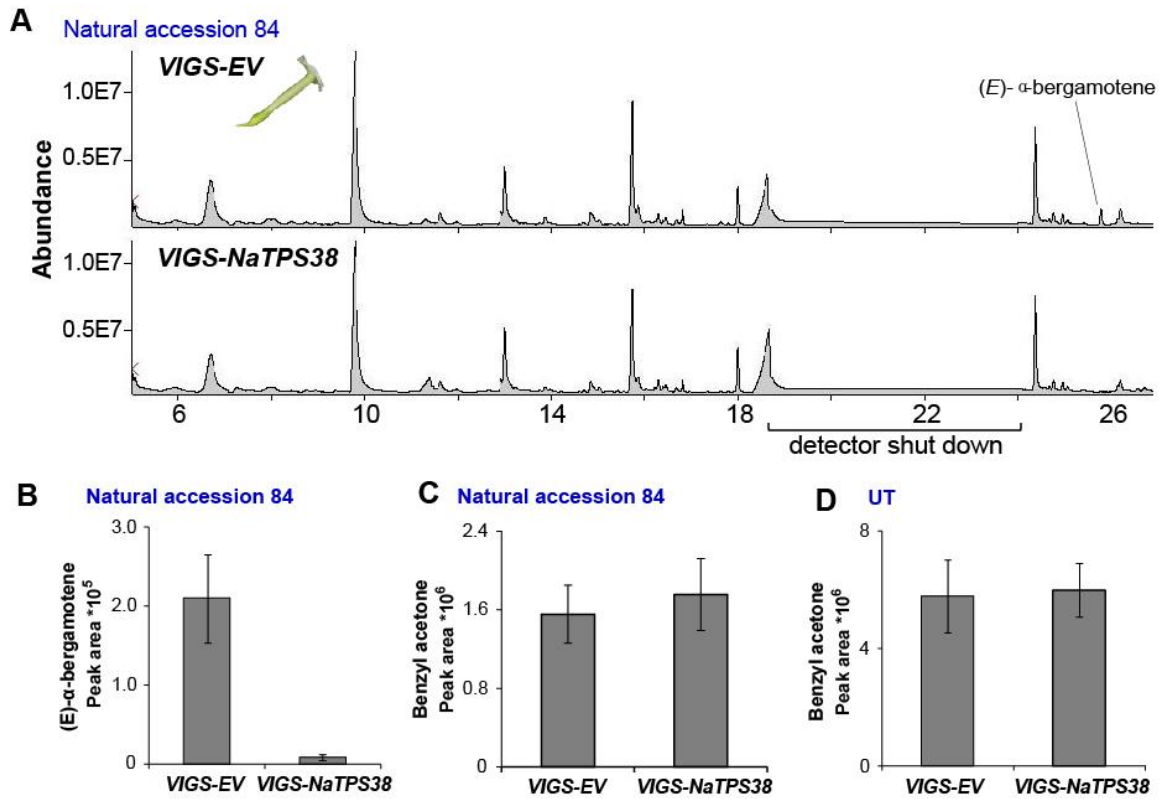


Figure S4. Silencing *NaTPS38* specifically reduced (*E*)- α -bergamotene emission in *N. attenuata* flowers, related to Figure 4.

A and **B**, silencing the expression of *NaTPS38* using VIGS specifically reduced emission of (*E*)- α -bergamotene, but not other volatiles in flowers. To prevent the overloading caused by the high amount of benzoyl acetone, the detector was shut down during the indicated retention time window. **C** and **D**, using a higher split ratio (1:10), we analyzed the benzyl acetone level from another PDMS tube used to sample the same flower (C-D). In comparison to VIGS-empty vector (EV) flowers, levels of benzyl acetone emission in flowers of VIGS-*NaTPS38* plants were not changed ($P > 0.1$, Student's-*t* test). To control the variation that might be caused by a different genomic background, the VIGS experiments were performed in two independent genotypes, UT and 84.

Table S1. Estimated effect size of identified QTL loci, related to Figure 2.
 The QTL identifiers are indicated in Figure 2. SE refers to standard error.

		QTL1	QTL2
Linkage group		LG1	LG2
Position (cM)		90.69	296.95
Additive effect	SE	0.02	-0.16
	<i>P</i> value	0.95	0.02
	Estimate	1.16	<0.001
Dominant effect	SE	0.21	-0.20
	<i>P</i> value	<0.001	0.09

Table S2. TPS38 orthologue genes in different diploid solanaceous species, related to Figure 3.

Species	Gene ID	Data source
<i>Petunia axillaris</i>	Peaxi162Scf00931g00514	[S2]
<i>Solanum melongena</i>	Sme2.5_05438.1_g00002	[S3]
<i>Capsicum annuum</i>	CA02g17550	[S4]
<i>N. tomentosiformis</i>	NITOMv2_g07214	[S5]
<i>N. obtusifolia</i>	NIOBTv3_g07214	[S6]
<i>N. sylvestris</i>	NISLVv2_g07214	[S5]
<i>N. attenuata</i>	NIATv7_g07214	[S6]

Table S3. The response of *M. sexta* antenna and proboscis to (*E*)- α -bergamotene, related to Figure 4. (*E*)- α -bergamotene elicited only a weak response on the antenna of *M. sexta* at relevant amounts, but was also detected by olfactory sensilla on the moth proboscis. The response of *M. sexta* antenna to (*E*)- α -bergamotene was measured using electroantennograms (EAG), the response of the proboscis sensilla was measured using single-sensillum recordings (SSR).

Antenna EAG			
Compound	Sample size	Mean EAD amplitude [μV]	S.E.M
Benzylacetone (2.98 μ g)	5	516	73.6
(<i>E</i>)- α -bergamotene (0.1 μ g)	5	112	30.1
Solvent control	5	60	17.9
Proboscis SSR			
Compound	Sample Size	Δ Spikes/s	S.E.M
Benzylacetone (2.98 μ g)	3	22.5	2.95
(<i>E</i>)- α -bergamotene (0.1 μ g)	4	15.5	2.48
Solvent control	4	1.75	1.25

Table S4. The kinetics of floral (*E*)- α -bergamotene emission from the natural accession 84 and the AI-RIL line 24A, related to Figure 4.

The emission was measured at three time points: 8-9 pm, 10-11 pm, 2-3 pm. Data are shown as mean \pm SD.

Genotype	<i>(E)</i> - α -bergamotene (peak area)		
	8 -9PM	10-11 PM	2-3 AM
84	400,549 \pm 112,740	190,231 \pm 32077	155,462 \pm 130,339
24A	89,648 \pm 22,217	123,204 \pm 36908	133,152 \pm 127,914

Supplemental Experimental Procedures

Plant material

Seeds of *Nicotiana attenuata* Torrey ex. Watson (Solanaceae) natural accessions were collected by Ian T. Baldwin and collaborators in the southwestern United States and inbred for one generation in the glasshouse. Two genotypes, UT and AZ used for developing the mapping populations, have been self-fertilized for 30 and 22 generations in the glasshouse, respectively. To develop the AI-RIL population, UT and AZ were first crossed to generate F1 plants, which were then self-fertilized to generate 150 F2 plants. From F2 to F6, in each generation, we intercrossed ~150 progeny using a random mating and equal contribution crossing design [S7]. For generation F7, two seeds from each of the crosses at F6 were germinated and used for the single-seed descendent inbreeding process. In total, five generations of inbreeding were conducted.

Plant growth conditions

All seeds were germinated following the protocol described by Krügel *et al.* [S8]. Plants were grown under in glasshouse conditions ($26 \pm 1^\circ\text{C}$; 16h : 8h, light: dark) [S8]. For the VIGS and subcellular localization experiments, plants were grown in climate chamber under a constant temperature of 26°C and 16h:8h (light: dark) light regime, and 65% relative humidity [S9].

Measuring kinetics of herbivory-induced leaf, and floral (E)- α -bergamotene emission

We measured the emission kinetics of wounding and *Manduca sexta* oral secretion (OS)-induced (*E*)- α -bergamotene in leaves of the 30th generation inbred UT plants at the rosette stage (35 d after germination). OS was collected on ice from *M. sexta* larvae reared on *N. attenuata* plants as previously described [S10]. To simulate herbivore attack, one stem leaf of each plant was wounded with a pattern wheel and 20 μL of 1:5 diluted OS was added to the puncture wounds at 8 am. After the OS treatment, the leaf was immediately enclosed into a plastic cup (ethylene terephthalate, ~300 mL), and three polydimethylsiloxane (PDMS) tubes were incubated into the cup to absorb the plant volatiles emitted from the leaf as described by Kallenbach *et al.* [S11]. To obtain temporally resolved quantitative data for (*E*)- α -bergamotene, the PDMS tubes were collected and replaced by three new ones at 4 h (12 pm), 8 h (4 pm), 12 h (8 pm), 16 h (12 pm), 20 h (4 am), 24 h (8 am), 28 h (12 am), and 32 h (4 pm in the next afternoon) after simulated herbivore attack, and kept in -20°C until analysis. For each collection, the uninduced leaves from intact plants were used to measure constitutive emission levels of (*E*)- α -bergamotene.

We measured the emission kinetics of floral (*E*)- α -bergamotene in flowers of both UT and AZ plants (50 d after germination). As flowers of *N. attenuata* remain open for three consecutive days and the flower age affects the quantity of floral volatiles, in the morning (8 am - 9 am) of the day for the volatile trapping, all flowers which opened the day before were removed. For each biological replicate, two flowers of the same plant were taken at 6 pm, 8 pm, 10 pm, 12 pm, 2 am, 6 am, 10 am and 2 pm and placed into a 15 mL glass vial (Sigma), where two PDMS tubes were placed ~2 cm away from the corolla limb before the vial was sealed. The PDMS tubes were collected at 1 h after incubation and kept in -20°C until analysis.

To further determine whether (*E*)- α -bergamotene was emitted from the inside or the outside of the corolla tube, we measured the floral bouquet of the whole flower as well as the bouquet of the corolla tube from the outside. For the bouquet of the whole flower, one flower was completely placed into a sealed glass vial (15 mL) together with a PDMS tube. For measuring the bouquet from the outside of corolla tube, the flower was inserted into the glass vial through a hole in the cap and the joint between the corolla tube and the hole was further sealed using a parafilm membrane to reduce the penetration of volatiles. In this setup, the whole corolla tube was inside the glass vial while the corolla limb and the corolla tube mouth were outside of the glass vial. Two PDMS tubes were placed into the glass vial for volatile collection for 1 h (between 8 pm and 9 pm) and the collected PDMS tubes were kept in -20°C until analysis. The results showed that the outside of corolla tube only produced 4.9% of total floral (*E*)- α -bergamotene (peak area of whole flower bouquet: $779,498 \pm 53,775$, peak area of bouquet produced by outer corolla tube: $38,349 \pm 14,903$).

Measuring floral and herbivory-induced leaf (E)- α -bergamotene among 23 natural accessions

The same treatment and sampling method as mentioned above was used to measure both floral and herbivore-induced (*E*)- α -bergamotene from plants from 23 natural accessions. To measure herbivore-induced (*E*)- α -bergamotene emission, all plants (50 d after germination) were treated with an OS elicitation at 8 am and volatiles were trapped between 12 pm and 4 pm using PDMS tubes. To measure floral (*E*)- α -bergamotene emission, one flower from each plant (60 d after germination) was collected at 9 pm and 10 pm and placed into a glass vial together with two PDMS tubes. At the end of sample collection, both herbivore-induced leaves (at 4pm) and flowers (at 10 pm) were immediately flash frozen in liquid nitrogen and then kept at -80 °C until gene expression analysis.

Sampling (E)- α -bergamotene in AI-RILs

The herbivore-induced (*E*)- α -bergamotene emission of AI-RIL plants (55 d after germination) was measured similarly as described above. The elicitation by OS was performed between 8-9 am, and two PDMS tubes were placed in the cup and incubated with the induced leaves for 24 h. The collected PDMS tubes were then kept in -20 °C until analysis. In total, (*E*)- α -bergamotene was measured in 256 samples.

Floral and herbivory-induced leaf volatile analysis by TD-GC-MS

PDMS pieces were placed in TDU autosampler tubes for TD-GC-QMS analysis on a TD-20 thermal desorption unit (Shimadzu) connected to a quadruple GC-MS-QP2010Ultra (Shimadzu). Specifications for desorption, columns used, and the spectra reading and identification, were as described by Kallenbach *et al.* [S11] and Schuman *et al.* [S12]. For all of the herbivory-induced volatiles, a 1:20 split was used, while no split was used for floral volatile analysis. To avoid overloading the detector due to the high abundance of benzyl acetone (BA) and nicotine, for the floral volatile analysis, we specifically shut down the detector at the retention times of the BA and nicotine peaks. The abundance of BA and nicotine were then analyzed using the second PDMS tube collected from the same sample using the same method but with 1:10 split.

QTL mapping

The genotype information of all AI-RIL plants and the linkage map were obtained from the dataset reported earlier (Xu *et al.* in revision). The R package QTLRel was used for QTL mapping following the tutorial [S13]. Briefly, the relationship among different individuals was first estimated based on pedigree information. The peak area of each compound was log-transformed. Samples with missing genotype or phenotype information were removed. In total, 207 samples were used for QTL mapping. Then the variance of the traits within the population was estimated via “estVC” and missing information of the genotypes was imputed using function “genoImpute”. The estimated trait variance and imputed genotypes were then used for the genome-wide scan. The empirical threshold was estimated based on 500 permutations. The additive and dominant effects of the candidate QTLs were estimated by fitting a multiple QTL model using the function “gls”.

Measuring expression of NaTPS38 from 23 natural accessions

The expression of both floral and herbivory-induced *NaTPS38* was measured in the same samples that were used for measuring (*E*)- α -bergamotene emission using qPCR. Total RNA was isolated using TRIzol reagent (Invitrogen) according to the manufacturer’s protocol. RNA samples were then treated with DNase-I (Fermentas) to remove genomic DNA. In total, ~1 μ g total RNA from each sample was reverse transcribed into cDNA using SuperScript II reverse transcriptase (Thermo Fisher Scientific). The relative transcript accumulation of *NaTPS38* was measured using qPCR on a Stratagene MX3005P PCR cyclor (Stratagene). For all qPCRs, the elongation factor-1A gene, NaEF1a (accession number D63396), was used as the internal standard for normalization [S14]. Forward and reverse primers used for qPCR are AGGGACAGCTCCTTCTCAAA and GGAAAGAGGAATGGGTTCAA, respectively. The coding sequence of *NaTPS38* is submitted NCBI under accession number XM_019369632.

To determine whether *NaTPS38* is expressed, PCR amplification was performed with Phusion Green High-Fidelity DNA polymerase (Thermo) using 40 PCR cycles to enable the detection of rare transcripts. The forward and reverse primers used are CAATGGATCTTAGGAGGTCA and TCAGGAAAGAGGAATGGGTTCAA, respectively.

To investigate temporal dynamics in the transcript accumulation of *NaTPS38* in *N. attenuata* flowers, the transcript abundance was measured at three time points: 6pm, 10pm and 2pm of the following day using plants of UT genotype. While the *NaTPS38* transcript abundance increased from 6 pm (1.41 \pm

0.20) to 10 pm (2.60 ± 0.74), which is consistent with the observed (*E*)- α -bergamotene emission pattern (Figure 1), the highest transcript abundance was found at 2 pm of the following day (6.84 ± 0.74), the time point when (*E*)- α -bergamotene emission was low. The inconsistency between *NaTPS38* expression and (*E*)- α -bergamotene emission at 2 pm of the following day could result from several non-exclusive reasons: 1) post-transcriptional regulation might be involved in regulating the abundance and function of NaTPS38 protein during the day; 2), at 2 pm, *N. attenuata* flowers are mostly closed. However, most of (*E*)- α -bergamotene emissions were found to be emitted from the inner side of the corolla tube. Therefore, even when the *NaTPS38* proteins are highly abundant and (*E*)- α -bergamotene is produced by the corolla tube, the compound was not emitted to outside and was not measured by our method; 3) substrate availability is known to fluctuate diurnally and might also contribute to the disconnection between the expression of *NaTPS38* and floral (*E*)- α -bergamotene emission in *N. attenuata* [S15]. Certainly, the mechanistic regulation of *NaTPS38* and (*E*)- α -bergamotene emission would require additional investigations, and are beyond the scope of our manuscript.

Cloning and heterologous expression of NaTPS38

The complete open reading frame of *NaTPS38* was cloned from cDNA using the primer pairs tps38fwd/ (ATGGATCTTAGGAGGTCAGGAA/TCAGGAAAGAGGAATGGGTTCAA). The resulting PCR fragments were cloned into the vector pET200/D-TOPO® (Invitrogen, Carlsbad, CA, USA) and *E. coli* strain BL21 Codon Plus (Invitrogen) was used for heterologous expression. Expression was induced by addition of isopropyl-1-thio-D-galactopyranoside to a final concentration of 1 mM. The cells were collected by centrifugation at 4000g for 6 min, and disrupted by a 4×30 sec treatment with a sonicator in chilled extraction buffer (50 mM MOPSO, pH 7.0, with 5 mM MgCl₂, 5 mM sodium ascorbate, 0.5 mM PMSF, 5 mM dithiothreitol and 10% v/v glycerol). Cell fragments were removed by centrifugation at 14,000 g, and the supernatant was desalted into assay buffer (10 mM MOPSO, pH 7.0, 1 mM dithiothreitol, 10% v/v glycerol) by passage through an Econopac 10DG column (BioRad, Hercules, CA, USA).

Enzyme assays were performed in a Teflon®-sealed, screw-capped 1 ml GC glass vial containing 50 μ L of the bacterial extract and 50 μ L assay buffer with either 10 μ M (*E,E*)-FPP or 10 μ M GPP, 10 mM MgCl₂, 0.2 mM NaWO₄ and 0.1 mM NaF. A solid phase microextraction (SPME) fiber consisting of 100 μ m polydimethylsiloxane (SUPELCO, Belafonte, PA, USA) was placed into the headspace of the vial for a 60 min incubation at 30°C and then inserted into the injector of the gas chromatograph for analysis of the adsorbed reaction products. GC-MS analysis was conducted using an Agilent 6890 Series gas chromatograph coupled to an Agilent 5973 quadrupole mass selective detector (interface temp, 250°C; quadrupole temp, 150°C; source temp, 230°C; electron energy, 70 eV). The GC was operated with a DB-5MS column (Agilent, Santa Clara, USA, 30 m x 0.25 mm x 0.25 μ m). The sample (SPME) was injected without split at an initial oven temperature of 50°C (monoterpenes) or 80°C (sesquiterpenes). The temperature was held for 2 min, then increased to 160°C (monoterpenes) or 200°C (sesquiterpenes) with a gradient of 7°C min⁻¹, and further increased to 300°C with a gradient of 100°C min⁻¹ and a hold of 1 min.

In addition, we also cloned and analyzed the enzymatic function of NaTPS25 using the same method mentioned above. The truncated version of *NaTPS25_AZ* (missing the first 13 codons) was amplified using primer pairs tps25fwd/rev. The result showed that NaTPS25 specifically produce (*E*)- β -ocimene (data not shown).

Measuring (E)- α -bergamotene emission from VIGS plants

The VIGS and leaf (*E*)- α -bergamotene measurements were carried out on the inbred line UT for *NaTPS38*. The inoculation was performed at 25 days after germination, and volatile trapping was started at 20 days after the inoculation. Before the volatile trapping, three leaves were sampled before any treatment to test silencing efficiency using primers: GAAGTTCAAACCGAGCCTTG (forward) and GGATTCATGTTCTCTCTTTTGTGA (reverse). Three more leaves, at the -1, 0 and +1 positions (indicating fully expanded to expanding leaves), were treated with 75 μ g MeJA dissolved in 20 μ L lanolin paste. This paste was applied at the base of the leaves at 12 pm, and after 1 h, half of these leaves were sampled to test the silencing efficiency. The whole shoot was then enclosed for volatile collection. The volatile analysis was carried out as described by Schuman *et al.* [S16] with the following modifications: plants received clean air filtered through activated charcoal at a rate of 600 mL/min and volatiles were sampled by pulling air through poropak Q filters at a rate of 300 mL/min. Eluents were analyzed using a

liquid autosampler on a GC-MS-QP2010Ultra (Shimadzu). The methods for the processing of these samples follow the description in Kallenbach *et al.* [S11]. This provided a quantitative comparison between samples, so the same eluents were also run on a Varian CP-3800 GC-FID for more quantitation. The methods for separation, processing, identification and quantification of volatiles for these samples follow those published by Schuman *et al.*[S12]. The qualitative and quantitative data sets collected were inspected graphically for normality and homogeneity and fit an appropriate statistical model in R (either *lm* or *gls*). Pairwise statistical comparisons of dependent variables were then extracted through *lsmeans*, from the R package *lsmeans*.

VIGS silencing efficiency and flower volatile measurements were also carried out in the inbred UT (30th generation) plants and an additional natural accession plants (84). Floral volatile trapping began 30 days after inoculation. On the day of volatile trapping, old flowers (opened before the trapping day) were removed in the morning (8 am-9 am). For each biological replicate, one flower was taken from the plant at 10 pm and placed into a 15 mL glass vial (Sigma), where two PDMS tubes were ~2 cm away from the corolla limb before the vial was sealed. PDMS tubes were collected after 1 h (11 pm) and immediately kept in -20 °C until analysis as described above.

Subcellular localization

The construction of 35S: *YFP-NaTPS25* and 35S: *YFP-NaTPS38* reporter fusions were carried out as described by Earley *et al.* [S17]. The open reading frame encoding *NaTPS25* and *NaTPS38* were amplified and introduced into pEarleyGate 104 to generate YFP fusion constructs. Primers used for cloning are: CACCATGGATCTTAGGAGGTCAGG (*NaTPS38* forward), TCAGGAAAGAGGAATGGGTTCAA (*NaTPS38* reverse), CACCATGCAGACACAAAAAGTTCA (*NaTPS25* forward) and AACGTGAATAGGCCGAGATT (*NaTPS25* reverse). Recombined plasmids were then transformed into *A. tumefaciens* strain GV3101 for subsequent plant transformation. Leaves of 3-weeks old *N. attenuata* were infiltrated with *A. tumefaciens* cells containing these constructs. To enhance the protein expression level of the terpene synthases, a P19 suppressor of gene silencing of *Tomato Bushy Stunt virus* (TBSV-P19) was co-expressed. YFP fluorescence was visualized 48 h following the inoculation with a Zeiss LSM 510 Meta confocal microscope (Carl Zeiss, Jena, Germany). The images were analyzed using LSM 2.5 image analysis software (Carl Zeiss, Inc.).

Molecular evolution of TPS38

We constructed a phylogenetic tree of the TPS-b clade using an in-house developed pipeline [S6, 18]. In brief, we aligned all coding sequences using MUSCLE (v.3.8.31) [S19] based on translated protein sequences with TranslatorX (v.1.1) [S20]. For all aligned sequences, all non-informational sites (gaps in more than 20% of sequences) were removed using trimAL (v1.4) [S21]. Then, PhyML(v. 20140206) [S22] was used to construct the gene tree with the best nucleotide substitution model estimated based on jModeltest2 (v.2.1.5) [S23] with the following parameter: -f -i -g 4 -s 3 -AIC -a. Support for each branch was calculated using the approximate *Bayesian* method.

The signature of positive selection on the branch of *TPS38* was estimated based on the ratio of non-synonymous (dN) and synonymous (dS) nucleotide substitution rate (ω) using PAML 4.7 [S24] package and ETE [S25] toolkit with the likelihood ratio test (LRT). Specifically, the branch of *TPS38* was marked as the foreground branch and the rest of branches were considered as the background branch. Then we performed the LRT test between the free-branch model (b_free), which assumes different selection pressure between foreground branch and background branch and the M0 model, which assumes the foreground and background branches are under the same selection pressure.

To investigate the signature of selection at the population level, we analyzed the nucleotide diversity among 23 genotypes of natural accessions (Xu *et al.* unpublished data) using a sliding window. In brief, for each accession, ~ 8 x whole genome resequencing data were obtained using Illumina HiSeq 2000. All of SNPs were called using the method described in Xu *et al.* (submitted). The neutrality test was based on the sliding window (window=1000bp, jump=100 bp) of Tajima's D, Fu and Li' F tests and minor allele site frequency spectrum (SFS) using the R package "PopGenome" (v2.1.6) [S26]. The genome-wide Tajima's D, Fu and Li's F and SFS are positive, suggesting either a population reduction in the past or that the unique fire chasing behavior of *N. attenuata* resulted in only a subset of genetic diversity were collected from a large seed bank.

Determination of the amount of (E)- α -bergamotene required for M. sexta moths Y-maze choice assay.

To determine the amount of (E)- α -bergamotene in the *M. sexta* moth choice assay, we measured and compared the (E)- α -bergamotene released from one flower from 9 pm to 10 pm (23 natural accessions) and a filter paper disc supplied with 10 μ L different concentrations of (E)- α -bergamotene in the 15 mL glass vial (Sigma). The concentrations of (E)- α -bergamotene (x) and peak area (y) detected from PDMS tubes correlated well ($R^2=0.9974$, $y=1E+08*x-207546$). This correlation allowed us to calculate the amount of (E)- α -bergamotene required to mimic the release from flowers among natural populations. The calculated (E)- α -bergamotene was then adjusted according to the volume of the container used for the Y-maze assay (50 mL), which resulted in 10 μ L 0.05 mM (E)- α -bergamotene, the concentration of which is similar to the levels of emission in the flowers of the natural assession 382 but lower than the emission from assession 84, 179 and 384.

Antenna and proboscis electrophysiology

Electroantennograms were performed by isolating the antenna of a three day old, male moth, which had been sedated at 5 °C. The two last flagellae on the tip of the antenna were also cut to increase conductivity and both ends of the antenna were placed into glass capillaries containing insect saline solution. The electrical responses of the antenna were recorded via Ag-AgCl wires, which were placed into the saline solution and connected to a ten times high impedance DC amplifier (Syntech, The Netherlands). The signal was then received by a digital/analog converter (Syntech, The Netherlands) and saved on a PC at a recording rate of 2.4 kHz. Odor stimuli were prepared by pipetting 10 μ L of the odorant onto a filter paper, which was placed into a glass pipet. The stimulus was then applied by running an air flow of 0.2 L/min through the pipet into a humidified air stream of 0.8 L/min that was constantly applied to the antenna.

Single sensillum recordings on proboscis of *M. sexta* have previously demonstrated an olfactory function of the mSt4 sensillum [S27]. Here we used the same technique, placing one tungsten electrode as a ground into the proboscis of an immobilized, three-day-old, male moth. A second tungsten electrode was then used to penetrate the mSt4 sensillum. Signal amplification, recording and stimulus delivery was performed as described for the electroantennograms. In contrast to a previous study, in which we did not show a response of the mSt4 sensillum to a mixture containing (E)- α -bergamotene, purified (E)- α -bergamotene was used in the present study. The results showed that the mSt4 sensillum is likely involved in the detection of (E)- α -bergamotene.

Proboscis choice assays

To examine the proboscis choice of *M. sexta* with (E)- α -bergamotene, we use a custom-built Y-maze system [S27]. For each assay, 10 μ L 0.05 mM (E)- α -bergamotene solution (dissolved in hexane) or pure solvent control (hexane) were pipetted onto a filter paper disc placed in a 50 mL glass bottle. Filtered clean air was pushed into the bottle and the air flow at each Y-maze arm attained 0.1 L/min. To prevent the moths' antenna from contacting (E)- α -bergamotene, the air was removed from the opening of the Y-maze at a rate of 0.2 L/min. The moths were allowed to forage freely for 4 min. A video camera (Logitech C615) was used to record the movement of the moth proboscis. The corolla limb from a freshly cut *N. attenuata* flower was attached to the opening end of the Y-maze to promote the vision orientation and improve the probing accuracy of the moth to the Y-maze system.

Using the corolla limb from the freshly cut *N. attenuata* flower did not affect the results we obtained for three reasons: 1) we installed an air-suction at the very front of the Y-maze, directly behind the attached corolla limb. In such a setup, none of the volatiles emitted from corolla limb enter into the Y-maze that was designed to test the proboscis responses; 2), in each test, the same corolla limb was used to measure the responses in the treatment as well as in the control. Given the large sample sizes of the analysis (N=19 for the single compound assay and N=21 for the whole floral bouquet of the VIGS-*NaTPS38* plants), it is unlikely that the observed pattern was influenced by the corolla limb by chance; 3) consistent patterns were observed for the experiments of the single compound assay and of the whole floral bouquet assay (repeated twice). These experiments were performed at different times using corolla limbs from different batches of *N. attenuata* plants. From these data, we conclude that using the corolla limb of *N. attenuata* did not influence the results we observed. Furthermore, because in nature, *M. sexta* moths are attracted by the BA emitted by the corolla limb of *N. attenuata* flowers (antenna response), our experimental setup closely reflects real pollination behavior of *M. sexta* moths.

To examine the proboscis choice of *M. sexta* under a whole floral volatile background, we compared whole floral bouquet of VIGS-*NaTPS38* plants and control plants (VIGS-EV) in the same Y-maze system.

The floral VOC from VIGS-*EV* and VIGS-*NaTPS38* flowers were examined prior the choice experiments, which showed that the silencing of *NaTPS38* specifically reduced the emission of (*E*)- α -bergamotene but not other floral VOCs (Figure S4A-B). The Y-maze setup and data analysis were same as the mentioned above. To approximately represent the natural concentration of (*E*)- α -bergamotene in the corolla tube, we used 10 undamaged fresh flowers for each assay, since the total volume of the glass bottle is much bigger than the volume of the corolla tube, without multiple flowers, the concentration of (*E*)- α -bergamotene would be diluted to much lower level than the one in the corolla tube of a single flower.

We performed *M. sexta* proboscis choice assay under whole floral volatile background twice using two different genotypes (UT and 84), which produce different levels of BA (high in UT, low in 84) and (*E*)- α -bergamotene (low in UT, high in 84). Although the magnitude of the effects on probing behavior of *M. sexta* differed between these two genotypes, likely due to different amount of (*E*)- α -bergamotene produced between these two genotypes, both experiments consistently showed that silencing *NaTPS38* reduced the probing time of *M. sexta*.

It is noteworthy that the concentrations of (*E*)- α -bergamotene used in the single compound proboscis choice assay and the whole floral bouquet choice assay are different. Due to the dilution effects explained above, the estimated (*E*)- α -bergamotene concentration that *M. sexta* moth accessed from the whole floral bouquet assay is only $\sim 1/50$ the concentration used for in the single compound assays. As higher concentration results in stronger effects (as seen from effect magnitude difference between UT and 84), the observed effect from our whole floral bouquet assay is a conservative estimate.

M. sexta moth pollination assay.

To test the influence of (*E*)- α -bergamotene on *M. sexta* pollination behavior under a semi-natural condition, we used the AI-RIL line 24A which emits very low levels of (*E*)- α -bergamotene in comparison to other AI-RIL plants. The floral (*E*)- α -bergamotene emission level of the accession 84, which produced the highest level of floral (*E*)- α -bergamotene among examined accessions, was set as the upper boundary. For all measurement, anthers of unopened new flowers were removed at 6 am (before anthesis) from the plants.

We then measured the emission difference between plants of the genotype 24A and 84 at three time points: 8-9 pm, 10-11 pm and 2-3 pm. The floral (*E*)- α -bergamotene level in accession 84 was 2.76-fold higher than that of 24A at 8-9 pm, then decreased to a similar level as 24A at 2 am (Table S4). Based on the observed differences, we tested the emission of (*E*)- α -bergamotene in flowers of 24A that were supplemented with different concentrations of (*E*)- α -bergamotene mixed with nectar. We found that adding 0.02 μ L (*E*)- α -bergamotene of a 1 mM stock in hexane mixed with 2 μ L nectar (with 0.04 μ L DMSO) to the flowers of 24A significantly increased the emission of (*E*)- α -bergamotene from the control (2 μ L nectar with 0.04 μ L DMSO and 0.02 μ L hexane) (Control: $95,188 \pm 26,822$, (*E*)- α -bergamotene supplementation: $224,474 \pm 15,220$) but these levels still remain lower than the emission levels found in flowers of accession 84 ($426,489 \pm 128,196$). Therefore this concentration was used for the *M. sexta* moth pollination assay in a tent in Jena between August and September 2016.

Data availability

The raw data for QTL mapping, kinetic of (*E*)- α -bergamotene emission in leaves and flowers and *M. sexta* probing assays are deposited in the figshare repository (<https://figshare.com/s/d0745f2a91ce77bbf5ac>).

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