Supporting Information

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SI Methods

Aphid Transcriptome Analysis. Microarray slides for analysis of *M. persicae* gene expression (1) were obtained from Agilent. One slide consists of eight microarrays, with each containing 15,744 features. Four arrays compared gene expression between Col-0–reared aphids treated with 1 μg EBF in hexane or hexane solvent–only control (comparison 1, Fig. 3*A*), and four arrays compared gene expression between Col-0- and EBFS-reared aphids both treated with 1 μg EBF (comparison 2, Fig. 3*A*). Similarly, gene expression changes in EBFS-reared aphids were studied upon treatment with 1 μg EBF (comparison 3, Fig. 3*A*), and on untreated aphids from both colonies (comparison 4, Fig. 3*A*). For each comparison, each array represented an independent biological replicates. Fifty aphids (fourth instars) from Col-0- and EBFS-expressing plants were transferred to a 50-mL tube and subsequently treated with 1 μg EBF or hexane for 30 min by applying a 5-μL droplet of EBF onto a filter paper. Aphids were harvested, frozen in liquid nitrogen, and stored at −80 °C until further processing.

Aphid RNA was extracted using the Ribopure kit (Ambion). Aphids were homogenized in 0.5 mL Tri Reagent (Ambion) by repeatedly drawing the liquid through a 21 gauge needle attached to a 1-mL syringe. RNA quantity and quality was assessed using the Nanodrop (Thermo Scientific) and Agilent bioanalyzers, respectively.

The Amino Allyl MessageAmp II Amplification kit (Ambion) was used to prepare RNA samples for array hybridization. RNA spike-ins (Two-Color RNA Spike-In kit; Agilent) were added to each sample. Reverse transcription was performed using a T7 oligo-dT primer. Following second strand synthesis and purification of cDNA, in vitro transcription was carried out with amino-allyl modified UTP. The resulting amplified RNA including amino-allyl modified nucleotides was used in a dye coupling reaction with the dyes Alexa Fluor 555 and 647. Purified amplified RNA was fragmented at 60 °C for 30 min and terminated by addition of 2× GEx Hybridization Buffer HI-RPM as described in the Agilent two-color microarray-based gene expression analysis protocol. Hybridization, wash, and scan of microarrays were performed according to the Agilent Two-Color Microarray-Based Gene Expression Analysis. Default Agilent scanner settings for 8 × 15K slide formats were used, and data were extracted using Agilent Feature Extraction software. The raw data from these experiments have been submitted to the Gene Expression Omnibus database of the National Center for Biotechnology Information.

Microarray data analysis was carried out using the LIMMA package within R (2–4). Within-array normalization was performed by the LOESS method without background subtraction. Linear modeling of arrays was performed with the lmFit function, and statistical analysis was performed using the eBayes function. Log fold-changes (LogFC) were computed and contigs with *P* values no greater than 0.05 were considered to be differentially expressed. A mean LogFC was used in cases in which multiple features representing a single contig were significantly altered (Dataset S1).

1. Ramsey JS, et al. (2007) Genomic resources for *Myzus persicae*: EST sequencing, SNP identification, and microarray design. *BMC Genomics* 8:423.
2. Smyth GK (2004) Linear models and empirical bayes methods for assessing differential expression in microarray experiments. *Stat Appl Genet Mol Biol* 3:3.
3. Smyth GK, Michaud J, Scott HS (2005) Use of within-array replicate spots for assessing differential expression in microarray experiments. *Bioinformatics* 21:2067–2075.
4. Smyth GK, Speed T (2003) Normalization of cDNA microarray data. *Methods* 31: 265–273.

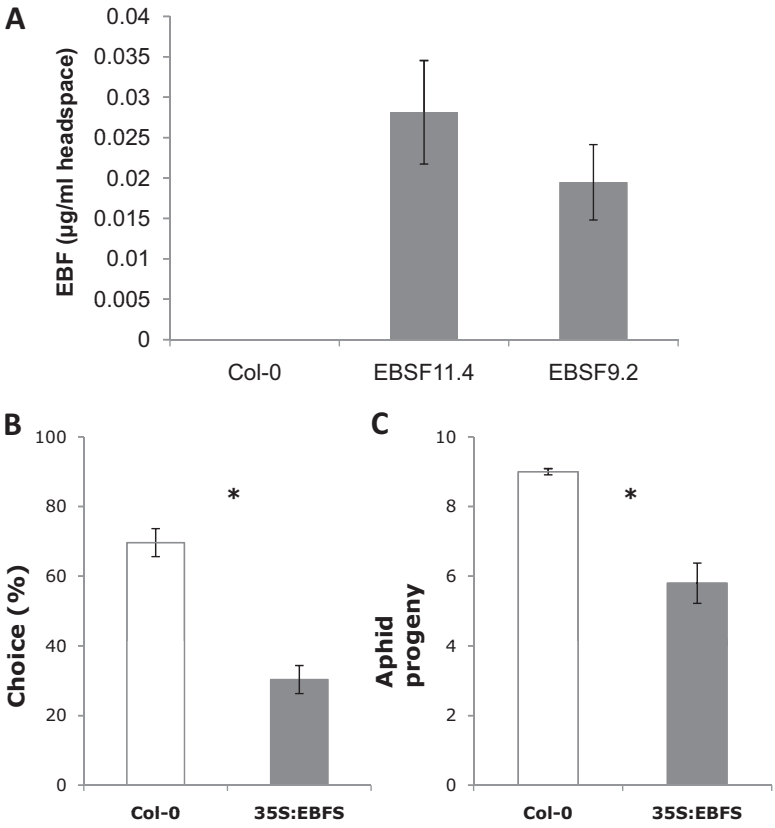

Fig. S1. (*A*) EBF in the headspace of whole Arabidopsis. Both 35S:EBFS transgenic lines used in these experiments emit EBF, but EBF is not detectable in the headspace of WT Col-0. Mean ± SE of $n = 5$. (*B* and *C*) Response of Col-0-reared *M. persicae* to WT (Col-0) and EBF-producing (35S:EBFS) *A. thaliana* plants (line 9.2 in ref. 1, a different line from line 11.4 that is shown in Fig. 1 *A* and *B*). (*B*) Choice tests, mean ± SEM of $n = 25$; $^*P < 0.05$, generalized linear model with a binomial error structure. (*C*) Aphid progeny production, mean ± SEM of $n = 12$; $^*P < 0.05$, generalized linear model with a Poisson error structure.

1. Beale MH, et al. (2006) Aphid alarm pheromone produced by transgenic plants affects aphid and parasitoid behavior. *Proc Natl Acad Sci USA* 103:10509–10513.

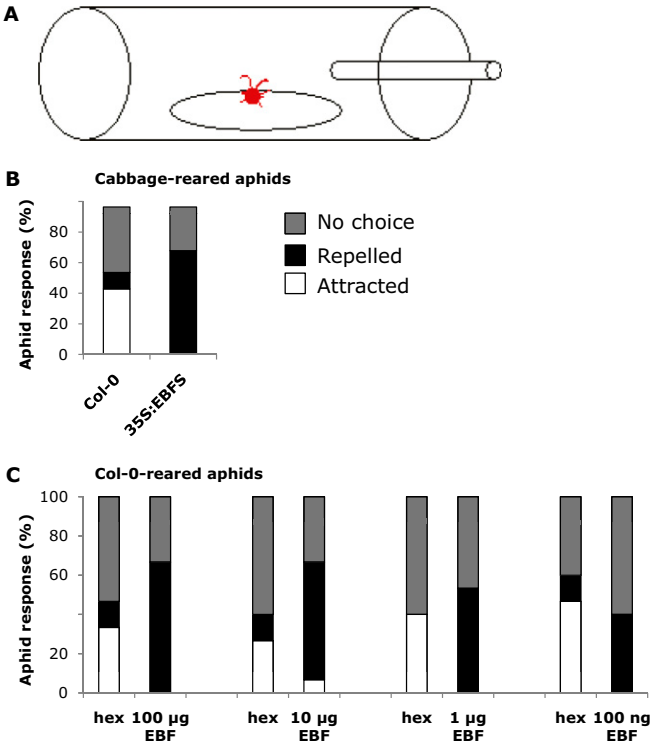

Fig. S2. (*A*) Drawing of the experimental setup used to determine aphid volatile responses. Aphids were placed on a filter paper disk in the center of a sealed 50-mL plastic tube and volatiles were introduced from one end with a syringe. (*B*) Cabbage-reared aphids are repelled more by 35S:EBFS than Col-0 volatile (n = 25; $P < 0.05$, Fisher exact test). (*C*) Col-0–reared aphids are significantly repelled by a thousand-fold range of synthetic EBF concentrations relative to solvent (hex, hexane) controls (n = 15; $P < 0.05$, Fisher exact test, for each pair-wise comparison).

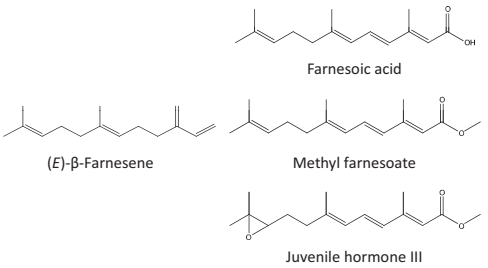

Fig. S3. Structural similarities between (*E*)-β-farnesene and three acyclic sesquiterpene juvenile hormones (farnesoic acid, methyl farnesoate, and juvenile hormone III) suggest a common metabolic origin in insects.

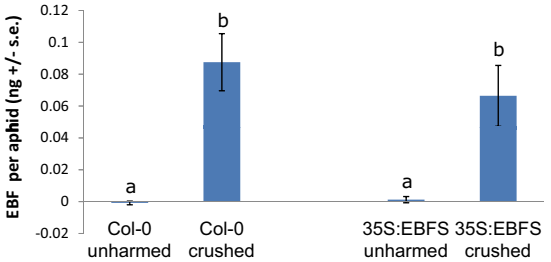

Fig. S4. EBF release in Col-0–reared and 35S:EBFS-reared aphids. Aphids were either undamaged or crushed to mimic a predator attack. Measurement of an empty-vial control was subtracted from each sample; mean ± SEM of n = 5. Different letters above the bars indicate significant differences ($P < 0.05$, generalized linear model with a Poisson error structure).

Other Supporting Information Files

Dataset S1 (XLSX)