Supporting Information

Huffaker et al. 10.1073/pnas.1214668110

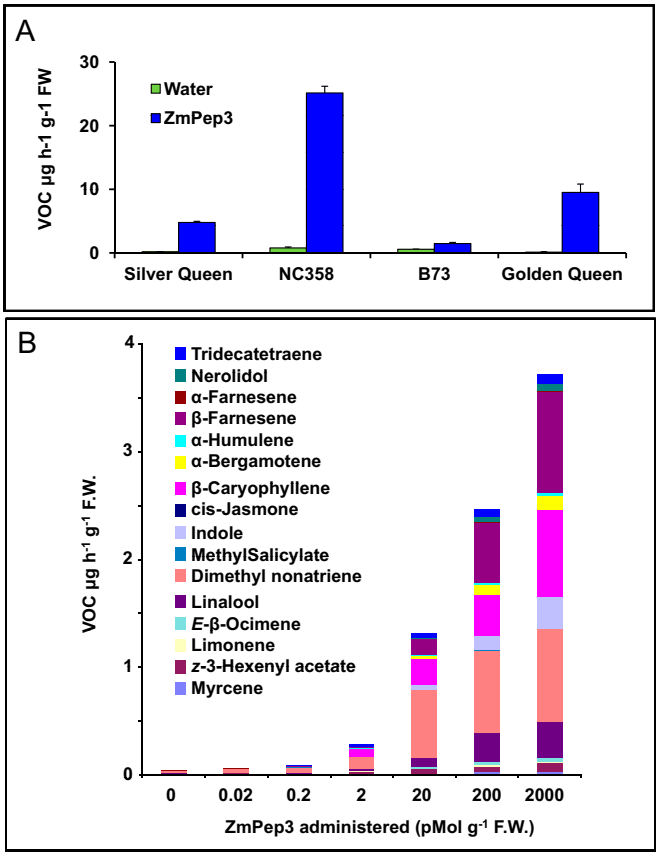

Fig. S1. *Zea mays* plant elicitor peptide 3 (ZmPep3) activates volatile emissions in multiple maize varieties and in a dose-dependent manner. (*A*) Total emitted volatiles from leaves 16 h posttreatment with water or with 2 nmol·g^{-1} fresh weight (FW) ZmPep3. NC358 and B73 are inbred varieties belonging to the nested-associated mapping population lines; Silver Queen and Golden Queen are commercially grown hybrid sweet corn varieties. (*B*) Total emitted volatiles from Golden Queen leaves 16 h posttreatment with water or with increasing quantities of ZmPep3. For each, mean ± SEM; $n = 4$.

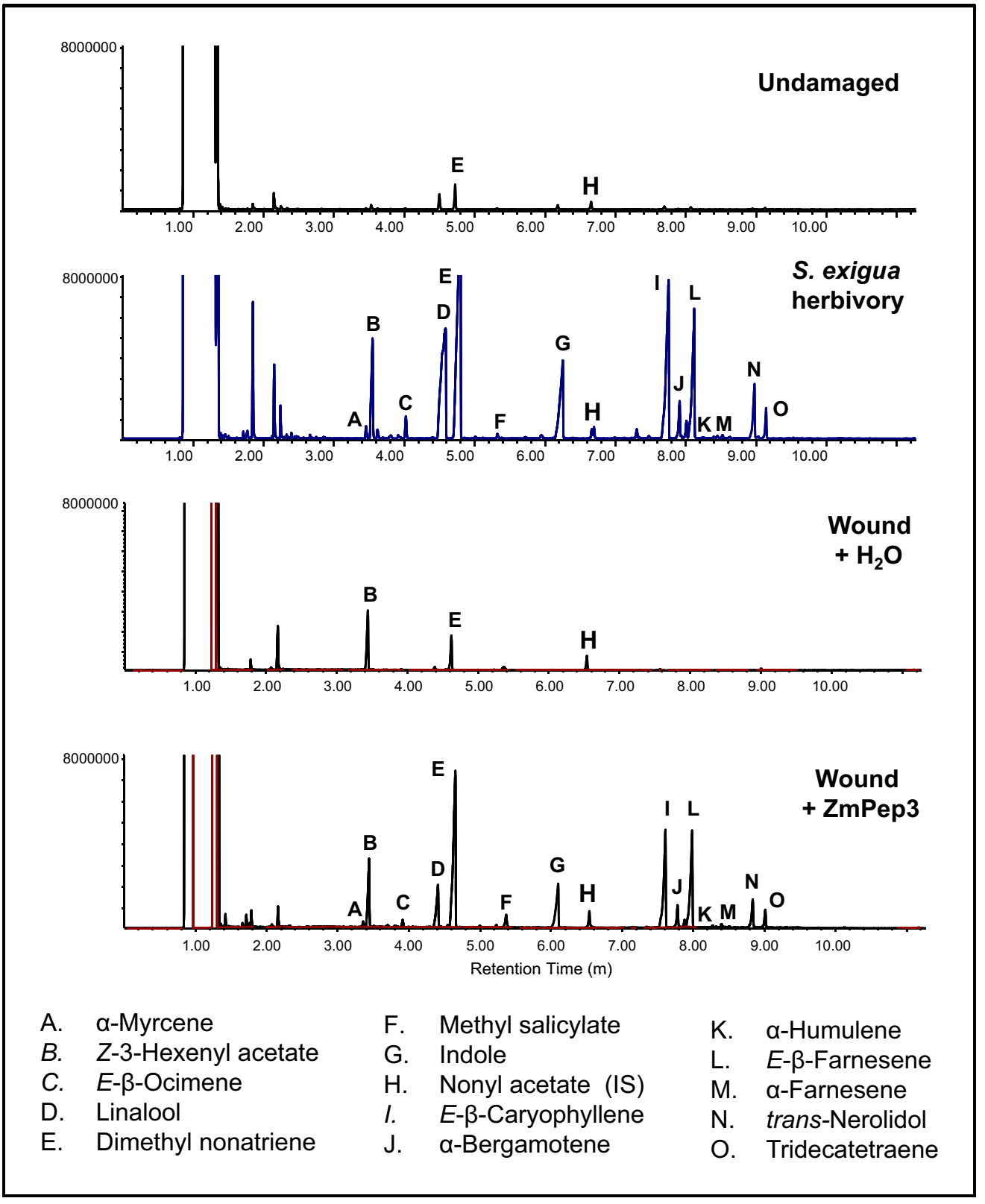

Fig. S2. Volatiles emitted by leaves treated with ZmPep3 are similar to those emitted by leaves subjected to *Spodoptera exigua* herbivory. GC-MS profiles of emitted volatiles undamaged leaves, from leaves 16 h postherbivory or posttreatment with water or with 2 nmol·g^{-1} FW ZmPep3, IS (H), internal standard. Maize variety used was Golden Queen.

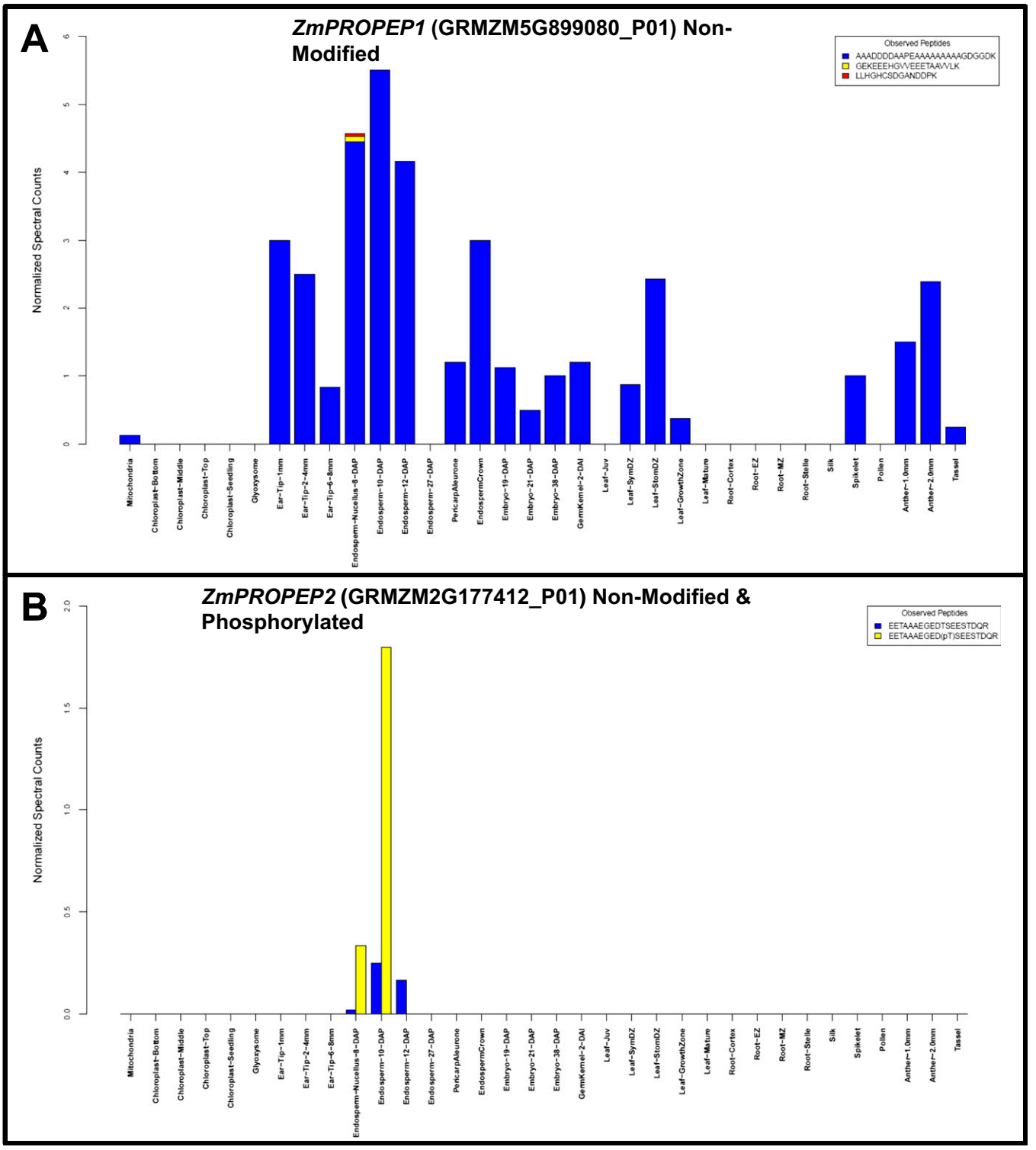

Fig. S3. ZmPROPEP protein abundance and phosphorylation observed across 34 distinct maize tissue types. (*A*) Three unique nonmodified peptides were observed from ZmPROPEP1. One peptide accounts for the vast majority of observations, and this peptide is seen across many different tissue types and developmental time points. No phosphorylated peptides were observed from this protein. (*B*) One unique peptide was observed from ZmPROPEP2 in both the unmodified and phosphorylated states. These peptides were only seen in the early endosperm. The phosphorylation site was 99% localized on the designated threonine residue with an average variable modification localization (VML) score of 1.24. No other ZmPROPEPs were observed in the dataset. Samples were harvested from maize B73 spanning multiple tissues, developmental time points, and organelles. Total proteins were extracted from each tissue and subjected to trypsin digest. Part of each sample underwent CeO_2 phosphopeptide enrichment. LC-electrospray ionization (ESI)-MS/MS runs were carried out on both phospho-enriched and nonenriched peptide samples with four biological replicates for each sample. Peptides underwent an on-line 72 h 2-dimensional (strong cation exchange/reverse-phase) chromatography run followed by nanoelectrospray ionization and tandem mass spectrometry on a Thermo LTQ Velos linear ion trap mass spectrometer. Database searches were done using Agilent Spectrum Mill MS Proteomics Workbench software. A maximum of one missed cleavage site was allowed as well as single or double phosphorylations. A concatenated forward-reverse protein database was constructed using the translated

Legend continued on following page

maize B73 5a genome and a 1:1 decoy database to calculate the false discovery rate (FDR). The peptide-level FDR was set to 0.1%. All validated peptide spectrum matches were summed to give spectral counts for each observed peptide in each run. The whole dataset was normalized by the total spectral counts for each run. Technical replicates were averaged for each biological replicate and then biological replicates were averaged to yield a final value for every peptide across all 34 sample types. Phosphorylation site localization was determined using Spectrum Mill's variable modification localization (VML) scoring (1).

1. Chalkley RJ, Clauser KR (2012) Modification site localization scoring: Strategies and performance. *Mol Cell Proteomics* 11(5):3–14.

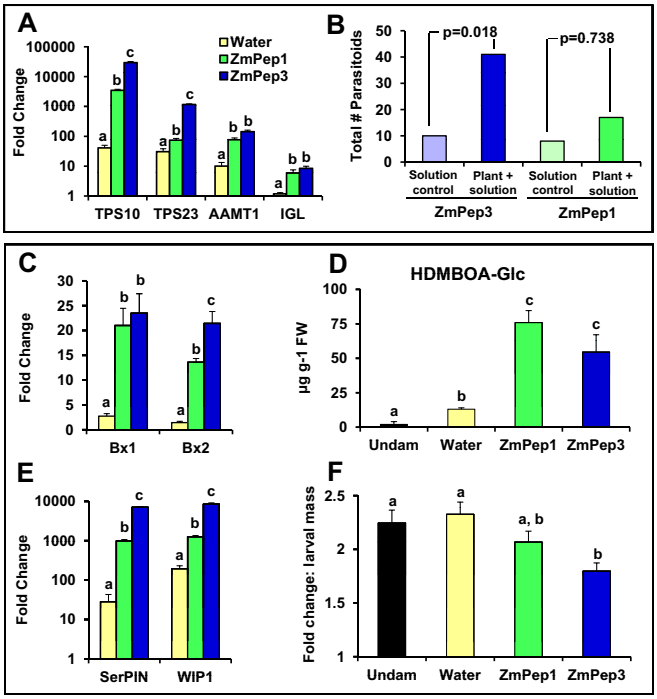

Fig. S4. Comparison of activities of ZmPep3 and ZmPep1 in eliciting antiherbivore defenses. (*A*) Relative expression 12 h posttreatment of genes encoding enzymes for the biosynthesis of the emitted volatiles, $n = 4$. (*B*) *Cotesia marginiventris* attraction to pretreated leaves (dark bars) or solution controls (slight bars) with ZmPep1 (green) or ZmPep3 (blue) each at 200 pmol·g^{-1} FW; representation of combined results from five replicate experiments. A four-arm olfactometer was used to compare the attractiveness of ZmPep1- and ZmPep3-treated plants as described (1). Five independent replicates were carried out, each consisting of 20–25 wasps that were released in groups of five. (*C*) Relative expression 12 h posttreatment of genes encoding biosynthetic enzymes for benzoxazinoid defense metabolites, $n = 4$. (*D*) Accumulation of the benzoxazinoid precursor 2-hydroxy-4,7-dimethoxy-1,4-benzoxazin-3-one glucoside (HDMBOA-Glc) 48 h posttreatment, $n = 4$. (*E*) Relative expression of genes encoding proteinase inhibitors after 12 h. (*F*) Fold change in *S. exigua* larval mass after 18 h on leaves that were either untreated (black) or pretreated for 48 h ($n = 24$). Statistics were used to compare plant tissues that were damaged and treated with either water, ZmPep1, or ZmPep3, applied at 2 nmol·g^{-1} FW. Within each plot, different letters (*a*–*c*) represent significant differences between mean values (± SEM; all ANOVAs, $P < 0.005$; Tukey test corrections for multiple comparisons, $P < 0.05$).

1. D'Alessandro M, Turlings TC (2005) In situ modification of herbivore-induced plant odors: A novel approach to study the attractiveness of volatile organic compounds to parasitic wasps. *Chem Senses* 30(9):739–753.

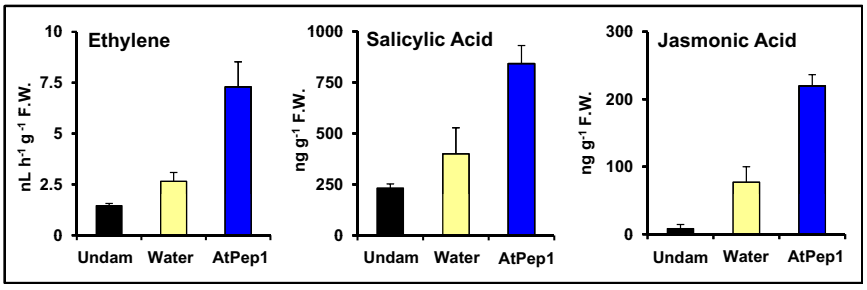

Fig. S5. In *Arabidopsis*, *Arabidopsis thaliana* plant elicitor peptide 1 (AtPep1) promotes production of the defense-related phytohormones ethylene, jasmonic acid, and salicylic acid. Phytohormone levels in undamaged (gray) *Arabidopsis* (Col.) leaves vs. leaves excised and treated with water (light blue) or a 50 nM solution of AtPep1 (dark blue). Ethylene was measured 1 h posttreatment, salicylic acid and jasmonic acid were measured 6 h posttreatment; mean ± SEM; $n = 4$.

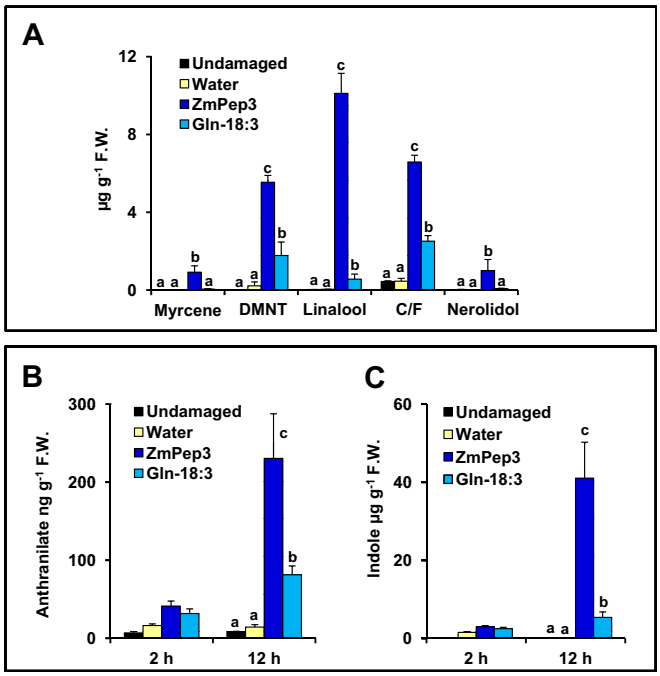

Fig. S6. Leaf metabolite pools following water, ZmPep3, or Gln-18:3 treatment. (*A*) Terpenes analyzed by vapor phase extraction coupled with GC/MS at 12 h. Axis label DMNT represents dimethylnonatriene, whereas C/F represents combined *E*-β-caryophyllene and *E*-β-farnesene, which cochromoatograph, (*B*) measured anthranilate and (*C*) indole at 2 and 12 h. All metabolites were quantified by GC/MS. Anthranilate pools represent the combined sum of free acid and methyl ester forms. For all experiments, leaves were treated with 2 nmol·g^{-1} FW ZmPep3 or Gln-18:3. Within each plot and analyte, different letters (*a*–*c*) represent significant differences between mean values ($n = 4$, ± SEM; all ANOVAs, $P < 0.01$; Tukey test corrections for multiple comparisons, $P < 0.05$).

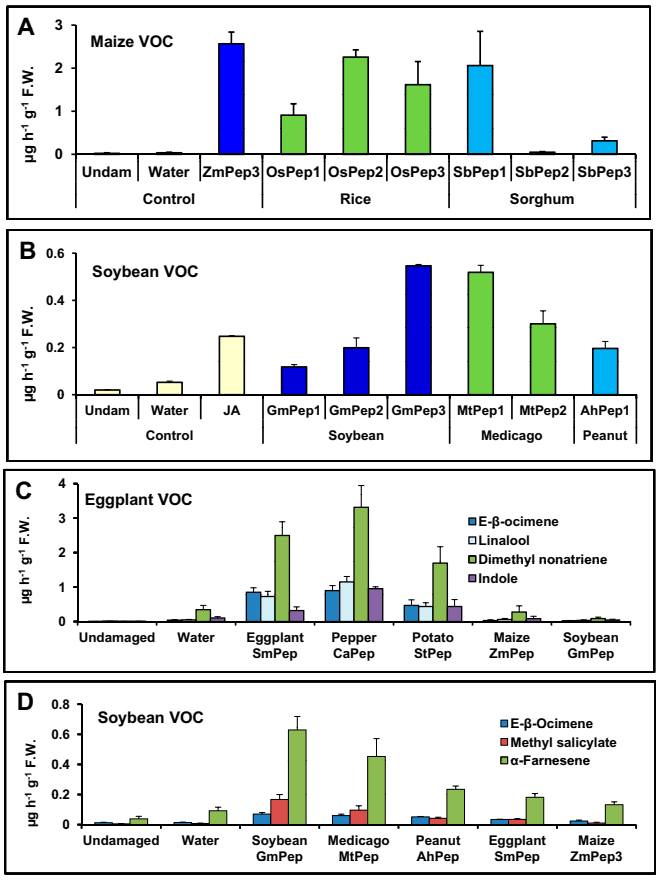

Fig. S7. Survey of Pep orthologs for volatile organic compound (VOC) emission-inducing activity and volatile components measured. *PROPEP*s are multigene families in most plants and the maize peptides display differential activity. Therefore, we screened for which Pep ortholog family members from other plants were capable of triggering VOC emission. Solanaceous orthologs were not screened to distinguish which family members were most active because in each Solanaceous species there is only one *PROPEP* gene. Measured volatiles were emitted from (*A*) maize leaves 16 h and (*B*) soybean leaves 5 h posttreatment with water or with 2 nmol·g^{-1} FW Peps. For each, mean; $n = 3$. Measured volatile components from (*C*) eggplant leaves or (*D*) soybean leaves 5 h posttreatment with water or with Peps.

Other Supporting Information Files

Dataset S1. Microarray analysis of genes expressed in response to ZmPep3 treatment

Dataset S1

Dataset S2. Peptides and primers used

Dataset S2