**Table X**. Effects of emitter damage treatment (control vs. herbivory by *Spodoptera exigua*), genotype, and their interaction on (a) total VOCs emission (linear mixed model, LMM) and (b) VOC composition (PERMANOVA) for *Arabidopsis thaliana* emitter plants from 24 different genotypes. In addition, we also show results from the LMM testing for effects of the emitter damage treatment, genotype, and their interaction on (c) the percentage of leaf damage from receiver plants previously exposed to emitter VOCs. We included size of plants as a covariate and population as a random factor. F-values/Pseudo-F for each factor, degrees of freedom and associated significance levels (*P*-value), as obtained from the corresponding models are shown. Significant *P-*values are highlighted in bold.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | a) Total VOC emissions | | | b) VOC composition | | | c) Leaf damage on receivers | | |
|  | Dfnum, den | F | *P* | Dfnum,den | Pseudo-F | *P* | Dfnum,den | F | *P* |
| Treatment (T) | 1, 100 | 1.606 | 0.208 | 1, 99 | 2.376 | **0.049** | 1, 107 | 13.287 | **< 0.001** |
| Genotype (G) | 23, 100 | 2.378 | **<0.01** | 23, 99 | 2.388 | **<0.001** | 23, 107 | 6.604 | **< 0.001** |
| T x G | 23, 100 | 0.346 | 0.997 | 23, 99 | 0.644 | 0.996 | 23, 107 | 0.69 | 0.845 |
| Plant size | 1, 100 | 1.958 | 0.165 | 1, 99 | 0.428 | 0.832 | 1, 107 | 0.255 | 0.614 |

**Figure 1**. Total emission of volatile organic compounds (VOCs, in nanograms per hour) emitted by *Arabidopsis thaliana* plants from 24 different genotypes in constitutive conditions (control) and herbivore-induced conditions (after *Spodoptera exigua* feeding). Bars are back-transformed least-square means ± SE from the LMM (N = 74–75).



**Figure 2**. Unconstrained ordination (PCoA) showing the effects of emitter induction treatment (control vs. herbivory by *Spodoptera exigua* feeding) on the composition of VOCs released by *Arabidopsis thaliana* plants from 24 different genotypes. Biplot arrows represent linear associations with the two most influential volatiles based on their R2 values scaled to reflect relative magnitude of effects. Diamonds represent the centroids for each emitter induction treatment and associated 95% ellipses. The first two axes together accounted for 58.09% of total variation in volatile composition due to differences among emitter damage treatment.



**Figure 3**. Unconstrained ordination (PCoA) showing the effect of *Arabidopsis thaliana* genotype (N = 74–75) on the composition of volatile organic compounds (VOCs) in (a) constitutive conditions (control) and (b) herbivore-induced conditions (after *Spodoptera exigua* feeding). Biplot arrows represent linear associations with the most influential volatiles based on their R2 values scaled to reflect relative magnitude of effects. The first two axes together accounted for 62.69% and 59.64% of total variation due to *Arabidopsis thaliana* genotype in constitutive and induced VOC composition respectively.



**Figure 4**. Effect of emitter induction treatment (control vs. herbivory-induced by *Spodoptera exigua*) on the percentage of leaf area consumed by *S. exigua* feeding on receiver *Arabidopsis thaliana* plants. Bars are back-transformed least-square means ± SE extracted from the linear mixed model (N = 78). Asterisks indicate significant differences between treatments (*P* < 0.001).



**Statistical analyses**

*Emitter treatment*

To test for genotypic variation on resistance level to *S. exigua* damage on induced emitter plants we ran a linear mixed model (LMM) testing for effects of *A. thaliana* emitter genotype (fixed factor) on the percentage of leaf area consumed. We square-root-transformed leaf damage to achieve normality of residuals.

*Effects of herbivory on emitter VOC emission.*

To test herbivory effects on quantitative variation in VOCs emissions we used linear mixed models (LMMs) testing for effects of emitter damage treatment (two levels: control or herbivory), *A. thaliana* genotype (24 levels), and their interaction (all fixed factors) on total VOCs released by emitter plants, as well as on each individual compound. In the case of individual VOCs, we performed *P*-value adjustments using the false discovery rate for *P* < 0.05 to avoid inflating Type I error due to multiple testing (Benjamini and Hochberg, 1995). We square-root-transformed VOCs emissions to achieve normality of residuals.

Additionally, to test for herbivory effects on qualitative variation in VOCs emissions, we ran a permutational multivariate analysis of variance (PERMANOVA) based on 10,000 permutations to test the effects of emitter damage treatment, *A. thaliana* genotype, and their interaction on VOC composition (using abundances of compound). To visualize these results, we conducted a principal coordinate analysis (PCoA) based on Bray-Curtis pairwise dissimilarities and graphed the centroids of each herbivory treatment effect (Moreira et al., 2021). We also identified the first two most influential VOCs as those having the strongest associations with the first two ordination axes based on their R2 values, and displayed these relationships using biplot arrows with the length scaled to R2 values.

For both the LMMs and PERMANOVA analyses above, we ran follow-up models testing for variation among *A. thaliana* genotype effect in VOCs separately for control and damaged plants to separately assess differences in emissions for pools of constitutive and induced VOCs, respectively. We square-root- and log-transformed VOCs emissions of control and damaged emitter plants, respectively, to achieve normality of residuals.

*Signalling effects on receiver plants.*

To test for signalling effects on receiver induced resistance, we ran a linear mixed model (LMM) testing for effects of emitter damage treatment (control or herbivory), genotype and their interaction (all fixed factors) on the percentage of leaf area consumed on receivers. The interaction was of special interest since it tested whether signalling effects in response to herbivory were contingent on the genotype. We also ran follow-up models testing for variation among *A. thaliana* genotype effect on leaf damage separately for receivers exposed to control and damaged plants to separately assess differences in resistance levels. We square-root-transformed leaf damage to achieve normality of residuals.

We ran all statistical analyses in R software version 4.3.0 (R Core Team, 2020). We implemented LMMs using the lmer function, from the lmerTest package (Kuznetsova et al., 2017). We report back-transformed least-square means and standard errors from these models using the lsmeans function from the lsmeans package (Lenth, 2016). In addition, we implemented PERMANOVA and ordination methods using the adonis and capscale functions respectively, both from the vegan package (Oksanen et al., 2016). In all cases, we included emitter or receiver plant size as a covariate to account for differences which could potentially affect volatile emissions or induced responses, respectively. We also included population as a random factor to control for source effects.

**RESULTS**

*Emitter treatment*

Analyses conducted on induced emitter plants did not show differences contingent to genotypic variation on resistance level to *S. exigua* damage (Table S1, Fig. S1).

*Effects of herbivory on emitter VOC emission*

We detected a total of 17 VOCs in the headspace of *A. thaliana* plants (Table S3). The LMM indicated significant effects of *A. thaliana* genotype (range: 7.56 ± 14.2 tetralin-equivalent ng h-1 to 241.25 ± 94.41 tetralin-equivalent ng h-1), but not of emitter induction (control = 50.62 ± 7.42 tetralin-equivalent ng h-1; induced = 62.59 ± 8.04 tetralin-equivalent ng h-1) or its interaction with *A. thaliana* genotype, on total VOC emissions (Table X, Fig. 1). However, analyses conducted separately for control and induced plants to further describe genotypic variation on VOC emissions did not indicate a significant effect of *A. thaliana* genotype on constitutive (F23,49 = 1.152, *P* =0.33) or induced (F23,50 = 0.609, *P* =0.847) total VOC emissions (Table S2). Similarly, analyses of individual compounds showed that there were no differences on VOC emissions between emitter treatments (Table S3).

The PERMANOVA indicated significant effects of emitter induction treatment and *A. thaliana* genotype, but not of their interaction, on VOCs composition (Table X). Emitter induction explained ca. 1.4% of the variation in VOCs composition, with the first two axes of the ordination together accounting for 58.09% of the variation due to this treatment (18.91% and 39.18%, respectively; Fig. 2). Variation in VOCs composition due to emitter induction was mainly associated with the relative amount of tridecane (R2 = 0.42, *P* < 0.001) and (E)-2-methyl-2-butenal (R2 = 0.41, *P* < 0.001). In addition, PERMANOVAs performed separately for control and induced plants to determine genotypic variation indicated a significant effect of *A. thaliana* genotype on the composition of both constitutive (DF= 23, 48, Pseudo-F = 1.59, *P* < 0.01) and induced (DF= 23, 49, Pseudo-F = 1.33, *P* =0.049) VOCs (Table S2. Fig. 3a, 3b). *A. thaliana* genotype explained 42.34% of the variation in the composition of constitutive VOCs, with the first two axes together accounting for 62.69% of the variation in VOCs due to this effect (18.09% and 44.6% respectively) (Fig. 3a). Variation due to *A. thaliana* genotype was mainly associated with the amount of tetradecane (R2 = 0.71, *P* < 0.001), dodecanal (R2 = 0.70, *P* < 0.001), tridecane (R2 = 0.76, *P* < 0.001), and (E)-2-methyl-2-butenal (R2 = 0.70, *P* < 0.001) (Fig. 3a). In addition, *A. thaliana* genotype explained 37.74% of the variation in the composition of induced VOCs, with the first two axes together accounting for 59.64% of the variation in VOCs due to this effect (19.68% and 39.96% respectively) (Fig. 3b). Compositional variation in induced VOCs due to *A. thaliana* genotype was mainly associated with the amount of (E)-2-methyl-2-butenal (R2 = 0.73, *P* < 0.001) (Fig. 3b).

*Signalling effects on receiver plants*

The emitter induction treatment significantly affected caterpillar consumption on receiver plants (Table X). Specifically, we found that the percentage of leaf area consumed by *S. exigua* on receiver plants exposed to damaged emitters was, on average, 38% lower compared to that of receivers exposed to control emitters (control = 23.92 ± 2.38%; herbivory = 14.99 ± 1.56 %) (Fig. 4). Moreover, there was a significant effect of receiver genotype on leaf herbivory, but not of its interaction with emitter induction treatment (Table X). Analyses conducted separately for receivers exposed to control and induced emitter plants to further describe genotypic variation on receiver resistance indicate a significant effect of *A. thaliana* genotype on receivers exposed to constitutive (F23,53 = 4.59, *P* <0.001) but not to induced (F23,18 = 0.012, *P* =0.911) VOC emissions (Table S4, Fig. S2).

**Supplementary Material**

**Table S1.** Genotype effect on the percentage of leaf damage by *Spodoptera exigua* feeding on *Arabidopsis thaliana* emitter plants from 24 different genotypes. We included size of plants as a covariate and population as a random factor. F-values for each factor, degrees of freedom and associated significance levels (*P*-values), as obtained from the corresponding models are shown.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Leaf damage on emitters | | |
|  | Dfnum,den | F | *P* |
| Genotype (G) | 23, 10 | 0.11 | 0.742 |
| Plant size | 1, 41 | 0.609 | 0.847 |

**Table S2.** Effects of genotype on (a, b) total VOCs emission (linear mixed models, LMMs) and (c, d) VOC composition (PERMANOVAs) for *Arabidopsis thaliana* emitter plants from 24 different genotypes subjected to a different emitter damage treatment: undamaged (control: a, c) or damaged by *Spodoptera exigua* feeding (herbivore-induced: b, d). We included size of plants as a covariate and population as a random factor. F-values/Pseudo-F for each factor, degrees of freedom and associated significance levels (*P*-values), as obtained from the corresponding models are shown. Significant *P*-values are highlighted in bold.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Total VOC emissions | | | | | | VOC composition | | | | | |
|  | a) Control | | | b) Herbivore-induced | | | c) Control | | | d) Herbivore-induced | | |
|  | Dfnum,den | F | *P* | Dfnum,den | F | *P* | Dfnum,den | Pseudo-F | *P* | Dfnum,den | F | *P* |
| Genotype (G) | 23,49 | 1.152 | 0.33 | 23,50 | 0.609 | 0.847 | 23, 48 | 1.589 | **< 0.01** | 23, 49 | 1.331 | **0.049** |
| Plant size | 1,49 | 1.681 | 0.201 | 1,50 | 0.11 | 0.742 | 1, 48 | 1.241 | 0.261 | 1, 49 | 1.172 | 0.298 |

**Table S3.** Means (± SE) for emission of individual volatile organic compounds (tetralin-equivalent ng h-1) identified by GC-MS under two emitter herbivore damage treatments (undamaged vs. damaged by *Spodoptera exigua*) in *Arabidopsis thaliana* plants 1. RT = Retention time. RI = Retention Index used for identification of compounds without commercial standards from the NIST database.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Compound** | **RT** | **RI** | **Control** | **Herbivory** | ***P-*value** |
| (E)-2-Methyl-2-butenal | 4.85 | 745 | 5.48±0.58 | 5.72±0.47 | 0.52 |
| α-Pinene | 9.48 | 937 | 3.76±0.67 | 3.89±0.59 | 0.53 |
| β-Pinene | 10.57 | 979 | 1.08±0.17 | 1.1±0.15 | 0.77 |
| 5-Methyl-2-heptanone | 10.78 | 971 | 2.79±0.30 | 2.54±0.24 | 0.56 |
| 6-Methyl-5-heptene-2-one | 10.85 | 986 | 10.56±2.16 | 10.21±1.37 | 0.45 |
| Butanoic acid, butyl ester | 11.06 | 995 | 1.28±0.21 | 1.33±0.18 | 0.33 |
| 2-Ethyl-1-hexanol | 11.84 | 1030 | 2.16±0.26 | 2.26±0.25 | 0.62 |
| Acetophenone | 12.76 | 1065 | 0.90±0.10 | 1.01±0.10 | 0.44 |
| Nonanal | 13.61 | 1104 | 10.29±2.09 | 9.83±1.19 | 0.42 |
| cis-2-Nonenal | 14.84 | 1148 | 0.90±0.13 | 0.91±0.12 | 0.58 |
| Dodecane | 15.67 | 1200 | 3.66±0.43 | 4.53±0.48 | 0.15 |
| Decanal | 15.82 | 1206 | 5.90±1.10 | 5.77±0.71 | 0.47 |
| Tridecane | 17.708 | 1300 | 2.89±0.34 | 3.83±0.46 | 0.07 |
| Tetradecane | 19.62 | 1400 | 3.03±0.41 | 5.41±1.01 | 0.03 |
| Dodecanal | 19.82 | 1409 | 9.36±1.15 | 10.41±1.15 | 0.44 |
| trans-Geranylaceone | 20.63 | 1453 | 4.72±1.05 | 4.69±0.71 | 0.49 |
| Tetradecanal | 23.36 | 1613 | 2.05±0.23 | 2.46±0.28 | 0.34 |

1We performed *P*-value adjustments using the False Discovery Rate for *P* < 0.05 to avoid inflating Type I error due to multiple testing.

**Table S4.** Genotype effect on the percentage of leaf damage by *Spodoptera exigua* feeding on *Arabidopsis thaliana* receiver plants exposed to (a) undamaged (control) or (b) damaged by *S. exigua* (herbivore-induced) emitter plants. We included size of plants as a covariate and population as a random factor. F-values for each factor, degrees of freedom and associated significance levels (*P*-values), as obtained from the corresponding models are shown. Significant *P*-values are highlighted in bold.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Leaf damage on receivers | | | | | |
|  | a) Control | | | b) Herbivore-induced | | |
|  | Dfnum,den | F | *P* | Dfnum,den | F | *P* |
| Genotype (G) | 23, 53 | 4.587 | **<0.001** | 23, 18 | 0.012 | 0.911 |
| Plant size | 1, 53 | 0.166 | 0.685 | 1, 18 | 2.546 | 0.32 |

**Figure S1.** Leaf damage by *Spodoptera exigua* on *Arabidopsis thaliana* emitter plants of 24 different genotypes. Bars are back-transformed least-square means ± SE extracted from the linear mixed models (N = 78). ****

**Figure S2.** Leaf damage by *Spodoptera exigua* on *Arabidopsis thaliana* receiver plants of 24 different genotypes exposed to (a) undamaged (control) or (b) damaged by *S. exigua* feeding emitter plants. Bars are back-transformed least-square means ± SE extracted from the linear mixed models (N = 78). Different letters over bars indicate significant differences between genotypes.

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