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Research

Leafminer attack accelerates the development of soil-dwelling conspecific pupae via plant-mediated changes in belowground volatiles

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Summary

- Herbivore population dynamics are strongly influenced by the interactions established through their shared host. Such plant-mediated interactions can occur between different herbivore species and different life developmental stages of the same herbivore. However, whether these interactions occur between leaf-feeding herbivores and their soil-dwelling pupae is unknown.
- We studied whether tomato (*Solanum lycopersicum*) leaf herbivory by the American serpentine leafminer *Liriomyza trifolii* affects the performance of conspecific pupae exposed to the soil headspace of the plant. To gain mechanistic insights, we performed insect bioassays with the jasmonate-deficient tomato mutant *def-1* and its wild-type, along with phytohormones, gene expression and root volatiles analyses.
- Belowground volatiles accelerated leafminer metamorphosis when wild-type plants were attacked aboveground by conspecifics. The opposite pattern was observed for *def-1* plants, in which aboveground herbivory slowed metamorphosis. Leafminer attack induced jasmonate and abscisic acid accumulation and modulated volatile production in tomato roots in a *def-1*-dependent manner.
- Our results demonstrate that aboveground herbivory triggers changes in root defence signalling and expression, which can directly or indirectly via changes in soil or microbial volatiles, alter pupal development time. This finding expands the repertoire of plant–herbivore interactions to herbivory-induced modulation of metamorphosis, with potential consequences for plant and herbivore community dynamics.

Introduction

Plants are attacked by multitudes of herbivorous arthropods (Stam *et al.*, 2014). Upon attack, plants can alter their primary and secondary metabolism to produce toxic or antinutritive substances that reduce herbivore preference, performance and/or survival (Mithöfer & Boland, 2012). These chemical readjustments are controlled by plant hormonal signalling, among which the jasmonate pathway is a central regulator (Erb & Reymond, 2019). Some herbivores, however, have developed intricate strategies to suppress or modify induced plant responses to their own advantage (Sarmento *et al.*, 2011; Robert *et al.*, 2012b; Chung *et al.*, 2013). Herbivore population dynamics are therefore strongly influenced by the interplay between the induction of plant chemical defences and herbivore manipulation of these defences (van Dam & Heil, 2011; Soler *et al.*, 2013; Kant *et al.*, 2015).

Plant defence induction and manipulation can influence the interactions between the plant and different life stages of the herbivore (Erwin *et al.*, 2014). Many herbivorous species complete their life cycle in different organs of the host plant, for example adults feeding on shoots and conspecific larvae feeding on roots. Initial leaf herbivory by adults can elicit systemic plant responses in the roots that facilitate or inhibit subsequent larval performance (Erwin *et al.*, 2014; Huang *et al.*, 2014; Kraus & Stout, 2019). While the impact of herbivory-induced plant defence responses on different plant-feeding life stages of conspecific and heterospecific herbivores have been studied extensively, little is known about their influence on nonfeeding life stages such as the pupal stage. This is surprising considering the large number of herbivorous arthropod species (including insects belonging to the orders Diptera, Hymenoptera, Thysanoptera, Coleoptera and Lepidoptera) that pupate in the soil close to their host and, thereby, their potential exposure to the chemicals released by the roots and their associated rhizosphere. In fact, as insect pupae are immobile, their survival, that is adult

emergence, is highly influenced by the soil habitat (Torres-Muros *et al.*, 2017).

As drivers of soil biotic and abiotic properties and producers of biologically active metabolites, plant roots play a key role in plant-mediated interactions between leaf- and root-feeding herbivores and their life stages (van Dam, 2009; Robert *et al.*, 2012a). Leaf herbivory can modulate the release of organic chemicals from roots, thereby shaping the surrounding habitat and the plant-associated soil communities (Barber *et al.*, 2012; Huang *et al.*, 2017; Karssemeijer *et al.*, 2020). In particular, root emission of volatile organic compounds (VOCs) can influence the behaviour of root herbivorous insects and their natural enemies (Rasmann *et al.*, 2005; Robert *et al.*, 2012b; Huang *et al.*, 2017) and potentially affect the physiology of the herbivore (Veyrat *et al.*, 2016; Ye *et al.*, 2018). Insect pupae can exchange gases through their spiracles (Jõgar *et al.*, 2007; Nestel *et al.*, 2007), it is therefore conceivable that leaf attack by herbivores may influence pupal development in the soil through systemic changes in the release of root volatile chemicals.

The American serpentine leafminer *Liriomyza trifolii* (Burgess) (Diptera, Agromyzidae) is a polyphagous insect herbivore that causes large economic losses of ornamental plants and vegetable crops worldwide (Minkenberg & Lenteren, 1986). Adults of *L. trifolii* are small flies (*c.* 2 mm long). Female flies feed and lay eggs inside the leaf, below the epidermis. The larvae subsequently feed within the leaf creating tunnels (mines). At the end of their third developmental stage, the larvae leave the mine and drop to the soil to pupate (Parrella *et al.*, 1983). Due to the rapid *L. trifolii* life cycle (12–24 d), plants infested with this leafminer can generally hold several generations of this insect, which means that adults, larvae and soil-dwelling pupae simultaneously occur in the field. *L. trifolii* is an important pest of cultivated tomatoes (*Solanum lycopersicum*) and can build up to high population densities during the growing season (Abe & Kawahara, 2001). Tomato roots are reported to produce and emit VOCs that influence the behaviour of soil-living nematodes (Murungi *et al.*, 2018). The *L. trifolii*–tomato system is therefore an ideal model to investigate if and how plants mediate interactions between leaf-feeding herbivores and their soil-dwelling pupae.

Very little is known about the interactions between *L. trifolii* and cultivated tomato at the molecular level (Stout *et al.*, 1994). Previous work in Arabidopsis suggests that plant resistance to *L. trifolii* mainly depends on jasmonate-regulated defence responses (Abe *et al.*, 2013). Here we performed insect bioassays to investigate whether *L. trifolii* aboveground infestation of tomato plants affects the performance of the leafminer pupae in the soil through changes in belowground volatiles. In addition, we investigated if these interactions depend on intact defence signalling through the use of the tomato mutant *defenseless-1* (*def-1*), deficient in the biosynthesis of jasmonic acid in response to wounding or herbivore attack (Li *et al.*, 2002). To gain insight into the chemical and molecular mechanisms underlying the tomato-leafminer interaction, we surveyed the accumulation of defence-related phytohormones, gene transcripts and root volatiles. Our results show that *L. trifolii* pupae develop faster when exposed to belowground volatiles from leafminer-infested tomato plants, and that these interactions are *def-1* dependent and partially explained by the induction of jasmonic acid (JA)-associated responses. Furthermore, *L. trifolii* infestation significantly altered the production of tomato root volatiles in a genotype-dependent manner. Together, our results indicate the modulation of pupal development in the soil following leaf infestation is plant mediated, and that JA and belowground chemistry play a relevant role in this interaction. These findings uncover herbivory-induced modulation of metamorphosis as a new phenomenon in plant–insect interactions.

Materials and Methods

Plant and insect materials

Tomato (*Solanum lycopersicum* Mill.) seeds of the jasmonate-deficient mutant *defenseless-1* (*def-1*), and its wild-type, cv 'Castlemart', were sown in plastic trays filled with potting soil in a climate room provided with 113.6 µmol m^2 s^{-1} of photosynthetically active radiation (PAR), a photoperiod of 16 h : 8 h, light : dark, 20°C, and 70% relative humidity (RH). At 15 d after germination, plantlets were transplanted to 13 × 13-cm plastic pots filled with the same potting soil.

A colony of the American serpentine leafminer *L. trifolii* (Burgess) (Diptera: Agromyzidae) was maintained on chrysanthemum plants in a climate room at 25°C and 70% RH.

Experimental design

For the experiments, 5-wk-old wild-type and *def-1* plants were individually placed inside transparent plastic cylinders (80 cm height, 20 cm diameter) closed at one end with a lid made of insect-proof gauze (Leiss *et al.*, 2009) (Fig. 1a, Supporting Information Fig. S1) and watered from the bottom every 2 d. Seven *L. trifolii* adult female flies were released inside each cage. Noninfested enclosed plants served as controls. Aboveground *L. trifolii*-infested and noninfested plants were placed randomly in a climate room provided with 113.6 µmol m^{-2} s^{-1} of PAR, a photoperiod of 16 h : 8 h, light : dark, 25°C and 70% RH. Aboveground infested plants were damaged by feeding and ovipositing *L. trifolii* adult flies and, subsequently, by the developing larvae that created tunnels (mines) within the leaves. At 8 d after infestation a custom-made cage containing 15 age-synchronised *L. trifolii* pupae (obtained from the rearing maintained on chrysanthemum; see above) was buried into the soil (*c.* 2 cm deep) close to the stem of each plant (Figs 1a, S1). Custom-made cages consisted of a Petri dish, sealed with parafilm, whose bottom lid was perforated and covered with an air-permeable but insect-proof mesh (Fig. 1a). Before the start of aboveground *L. trifolii* infestation treatment, aboveground and belowground compartments of the plant were separated by covering the soil with aluminium foil. This system therefore exposed the pupae to belowground VOCs only. The number of emerged adults per cage and plant was then recorded every 2 d for a period of 16 d. At the end of the experiment, that is, 24 d after infestation, the number of *L. trifolii* larvae-associated mines per plant and soil humidity were determined. In a second repetition of the

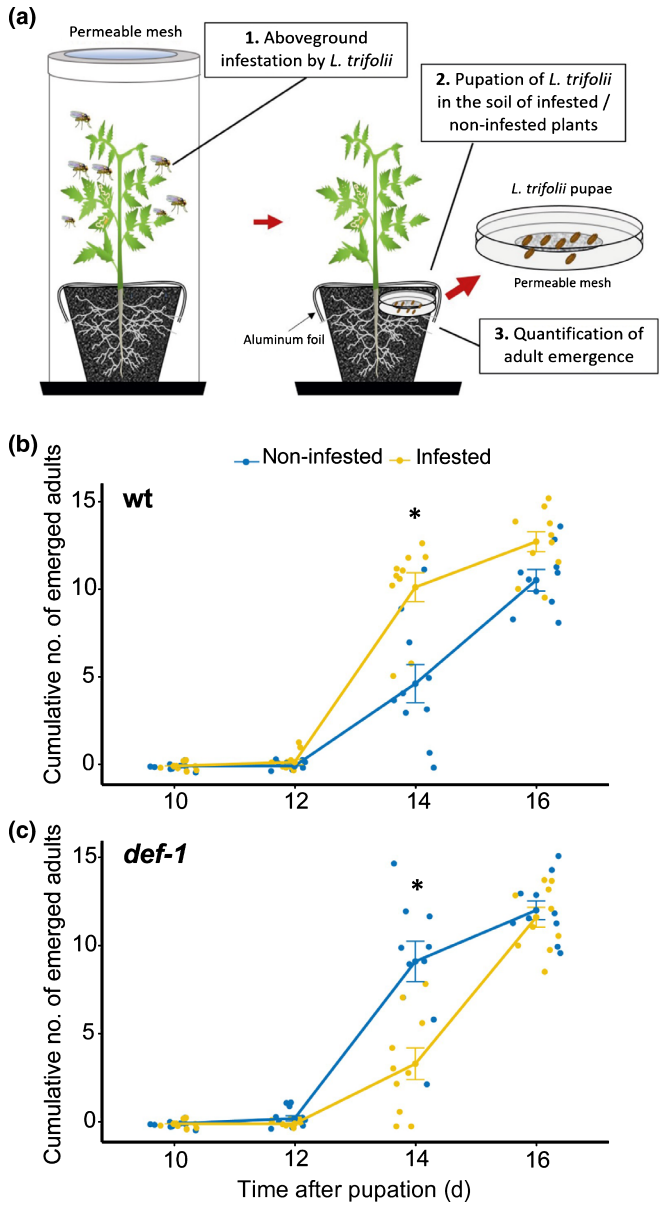

Fig. 1 Aboveground leafminer attack accelerates the developmental time of conspecific pupae in the soil in a *def-1*-dependent manner. (a) Overview of the set-up to test the effect of *Liriomyza trifolii* leaf herbivory on the performance of conspecific pupae in the soil. Here, 5-wk-old tomato (*Solanum lycopersicum*) plants were individually placed inside transparent plastic cylinders closed at one end with a lid made of insect-proof gauze and infested with seven *L. trifolii* adult female flies allowing them to feed and deposit eggs on the plants. Before *L. trifolii* infestation, the soil was covered with aluminium foil to separate the belowground and aboveground plant compartments. At 8 d after infestation, 15 age-synchronised *L. trifolii* pupae from an external rearing were confined to a custom-made and air-permeable cage and subsequently buried into the soil of each plant. The cumulative number of *L. trifolii* adults (mean ± SEM, $n = 10$) that emerged from pupae exposed to belowground volatiles from (b) noninfested or *L. trifolii*-infested wild-type (wt) plants and (c) noninfested or *L. trifolii*-infested *def-1* plants were recorded every 2 d for a total of 16 d. Data from panels (b) and (c) were obtained from the same experiment and statistically analysed altogether using a negative binomial model that included plant genotype, herbivory and their interactions as fixed effects, and plant unit as the random intercept. Asterisks denote significant differences tested by pairwise comparisons of estimated marginal means with the Tukey-adjusted *P*-value method ($P \leq 0.05$).

experiment, the effects of aboveground *L. trifolii* infestation on plant hormone concentrations, gene expression levels, root volatile contents, and root dry biomass were determined at 24 d after infestation, that is 16 d after pupation (experimental details given in the following paragraphs). Note that experiments with wild-type and *def-1* plants were carried out simultaneously and that the data were statistically analysed as such.

Soil humidity

Soil humidity was estimated using the gravimetric method (Parkin *et al.*, 2008). A 2-ml Eppendorf tube per plant and treatment ($n = 10$) was filled with fresh soil taken from the upper soil layer (i.e. 5 cm), weighed, oven dried at 60°C for 5 d, and re-weighed. Fresh and dry soil mass (g) were calculated by subtracting the weight of empty 2 ml Eppendorf tubes. Gravimetric water content (GWC) expressed as the mass of water per mass of dry soil was then calculated as follows: GWC = (wet soil (g) − weight dry soil (g))/weight of dry soil (g).

Phytohormone analysis

The concentrations of 12-oxo-phytodienoic acid (OPDA), JA–isoleucine (JA-Ile), salicylic acid (SA) and abscisic acid (ABA) were determined in the third leaf from the apex and in the roots of noninfested and infested wild-type and *def-1* plants at 24 d after aboveground *L. trifolii* infestation. For this, the phytohormones were extracted with ethyl acetate spiked with isotopically labelled standards (1 ng of d5-JA, 13C6-JA-Ile, d4-SA, and d6-ABA) and analysed using ultrahigh-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) following the procedures described in Glauser *et al.* (2014).

Gene expression analysis

Total RNA was isolated using TRI Reagent (Sigma-Aldrich) following the manufacturer's instructions. DNase treatment was carried out using gDNA Eraser (PrimeScript™ RT Reagent Kit, Perfect Real Time) following manufacturer's instructions (Takara Bio Inc., Kusatsu, Japan). Reverse transcription and first strand cDNA synthesis was performed using PrimeScript Reverse Transcriptase (TaKaRa Bio). For gene expression analysis, 2 µl of cDNA solution (the equivalent to 10 ng of total RNA) served as a template in a 10-µl quantitative reverse transcription polymerase chain reaction (qRT-PCR) using ORA™ SEE qPCR Mix (Axonlab) on an Applied Biosystems® QuantStudio® 5 Real-Time PCR system. The normalised expression (NE) values were calculated with the ΔCt method: $NE = (1/(PE_{target}\text{^}Ct_{target}))/(1/(PE_{reference}\text{^}Ct_{reference}))$ in which PE stands for primer efficiency and Ct for cycle threshold, as in Alba *et al.* (2015). The PEs were determined by fitting a linear regression on the Ct values of a

standard cDNA dilution series. Gene identifiers, primer sequences and references are listed in Table S1.

Root volatile analysis

Root volatiles from *L. trifolii*-infested and noninfested wild-type and *def-1* plants were collected and analysed using solid-phase micro-extraction–gas chromatography–mass spectrometry (SPME-GC-MS). For this, the whole root system was sampled, washed with tap water, gently dried with filter paper, and flash frozen in liquid nitrogen before analysis. Roots were then ground into a fine powder, and 100 mg were placed in a 20-ml precision thread headspace glass vial sealed with a screw cap made of magnetic silver and a septum of silicone/polytetrafluoroethylene (PTFE) (Gerstel GmbH & Co. KG). An SPME fibre (100 µm polydimethylsiloxane coating; Supelco, Bellefonte, PA, USA) was then inserted through the septum for volatile collection for 40 min at 50°C. Collected volatiles were thermally desorbed for 3 min at 220°C and analysed by GC-MS (Agilent 7820A GC interfaced with and Agilent 5977E MSD, Palo Alto, CA, USA). Desorbed volatiles were separated with splitless mode on a column (HP5-MS, 30 m, 250 µm ID, 2.5-µm film, Agilent Technologies, Palo Alto, CA, USA) with He as carrier gas and at a flow rate of 1 ml min^{-1}. A temperature gradient of 5°C min^{-1} from 60°C (hold for 1 min) to 250°C was used. Compound identification was based on similarity to library matches (NIST search 2.2 Mass Spectral Library, Gaithersburg, MD, USA) as well as retention time and spectral comparison with pure compounds. In addition to the SPME analysis, root volatiles were measured following a pentane extraction followed by GC-MS analysis. For this, tomato root volatiles were extracted from 200 mg of frozen ground root material in 1 ml pentane while subjected to vigorous shaking for 1 h. Pentane extracts were then centrifuged at 3050 ***g*** for 20 min, and 1 µl of pentane extract was injected into the GC-MS system (Agilent 7820A GC interfaced with and Agilent 5977E MSD, Palo Alto, CA, USA) using the pulsed splitless mode. Injected volatiles were separated and identified using the same column specifications and methods described above for the SPME desorbed volatiles.

Statistical analysis

Data analysis was conducted in R v.4.0 (R Core Team, 2016). We modelled the cumulative number of emerged adults with a negative binomial model using GLMMTMB package (Brooks *et al.*, 2017). Day of emergence, that is 12, 14 and 16 d (days when there were zero emerged adults for all treatments were not included in the analysis), plant genotype, herbivory and their interactions were included in the model as fixed effects, and plant unit as the random intercept. Residuals diagnosis, zero inflation and over dispersion tests were performed using the DHARMA package (Hartig, 2020). The significance of the fixed effects from the conditional model was tested via ANOVA type analysis (type II Wald chi-squared tests). Estimated marginal means (EMMeans), standard errors, confidence limits and significant differences between means with multiple comparison adjustments (Tukey's honest significant difference (HSD)) were estimated. Effects of herbivory, plant genotype and their interaction on hormone levels, gene expression, soil humidity, root dry biomass and levels of root volatile compounds were tested using two-way ANOVA. Pairwise comparisons of EMMeans with multiple comparison adjustments (Tukey's HSD) was used as a *post hoc* test. Before analysis, all residuals were tested for normality and equal variance using the Shapiro–Wilk and Levene's tests, respectively. Data that did not meet these assumptions were $\log_{10}$, squared root or Tukey's ladder of powers transformed before statistical analysis. The correlation between the cumulative number of emerged adults at 14 or 16 d after pupation and (1) the number of larvae per plant or (2) the soil moisture was determined by Pearson product moment and Spearman's rank correlation tests. A redundancy analysis (RDA) was conducted to explore root volatile profile differences among treatments following the methods described in Hervé *et al.* (2018). The RDA consisted of (1) fitting a multivariate linear regression between the chemical data and the controlled variables (genotype, herbivory and their interactions) and (2) performing two principal component analyses (PCA), the 'constrained PCA' which was applied on the fitted values of the regression and summarised the variation of the chemical data explained by the controlled variables and the 'unconstrained PCA', which was applied on the residuals of the regression and summarised the variation that is not related to the controlled variables. For this, data were first $\log(x+10)$ corrected for the presence of zeros and skewness, the means were centred to equals to zero, and the variables were scaled to have standard deviation equal to 1. To identify which factors significantly influenced the observed chemical variation in the 'constrained PCA' we subsequently performed RDA followed by permutation *F*-test (999 permutations) based on the canonical R^2 (Hervé *et al.*, 2018). Finally, we conducted a permutation *F*-test (999 permutations) to assess the influence of plant genotype, herbivory and their interaction on the chemical data, as well as the differences among groups by pairwise comparisons corrected with the false discovery rate method. These statistical analyses were performed and/or visualised using the R packages CAR, OLSRR, RCOMPANION, NLME, EMMEANS, GGPLOT2, GGPBUR, FACTOEXTRA, HOTELLING, VEGAN and RVAIDEMEMOIRE (Hervé, 2015; Mangiafico, 2015; Wickham, 2016; Fox & Weisberg, 2019; Hebbali, 2020; Kassambara & Mundt, 2020; Kassambara, 2020; Oksanen *et al.*, 2020; Curran & Hersh, 2021; Lenth, 2021; Pinheiro *et al.*, 2021).

Results

Leafminer herbivory accelerates pupal development in the soil

To test if aboveground infestation by the leafminer *L. trifolii* affects the performance of conspecific pupae in the soil, we infested wild-type tomato plants with *L. trifolii* and monitored pupal development over time in the soil of infested and noninfested plants (Figs 1a, S1). To monitor adult emergence, age-synchronised *L. trifolii* pupae were placed in Petri dishes and subsequently buried in the soil 8 d after aboveground leafminer

infestation. The Petri dishes were fitted at the bottom lid with a permeable nylon mesh that allowed for exchange of volatile chemicals between the rhizosphere and the pupae. At 14 d after pupation, two times more adults had emerged from batches of pupae that had been exposed to belowground volatiles from infested plants compared with noninfested plants (Fig. 1b; Tables S2, S3; day 14, wild-type noninfested vs infested $P \leq 0.001$, EMMeans pairwise comparisons). This difference was not significant at the end of the emergence period (Fig. 1b; Tables S2, S3; day 16, wild-type noninfested vs infested $P > 0.05$, EMMeans pairwise comparisons).

Acceleration of pupal development is *def-1* dependent

Induced plant defences, including those regulated by jasmonates, can mediate systemic shoot–root responses upon herbivore attack (Machado *et al.*, 2018). To gain insights into the potential involvement of JA-mediated signalling in leafminer-induced acceleration of pupation, we evaluated the impact of leafminer infestation on pupal development in the tomato mutant *def-1* (Fig. 1c). Importantly, while *def-1* plants are characterised by a reduced ability to mount JA-regulated defence responses following mechanical damage and/or herbivory (Lightner *et al.*, 1993; Howe *et al.*, 1996), growth-related traits (e.g. plant height, number of leaves and dry mass) are unaffected (Li *et al.*, 2002; Thaler *et al.*, 2002; Escobar-Bravo *et al.*, 2017). Compared with noninfested wild-type plants, leafminer pupae developed more rapidly in the soil of noninfested *def-1* plants (Tables S2, S3; day 14; *def-1* noninfested vs wild-type noninfested $P < 0.01$, EMMeans pairwise comparisons). Strikingly, aboveground leafminer infestation of *def-1* plants reduced rather than increased adult emergence at 14 d after pupation (Fig. 1c; Tables S2–S4; *genotype* × *herbivory*, $\chi^2 = 23.03$, df = 1, $P < 0.01$, ANOVA type II Wald chi-squared tests; *def-1* noninfested vs *def-1* infested $P \leq 0.001$, EMMeans pairwise comparisons). Leafminer adult emergence was similar in infested *def-1* and noninfested wild-type plants (Table S3; *def-1* infested vs wild-type noninfested $P > 0.05$, EMMeans pairwise comparisons). At the end of the experiment (day 16), no differences in adult emergence were observed between infested and noninfested *def-1* plants (Fig. 1c; Tables S2–S4; *genotype* × *herbivory* × *day*, $\chi^2 = 22.9$, $df = 1$, $P < 0.01$, ANOVA type II Wald chi-squared tests; day 16, *def-1* noninfested vs infested $P > 0.05$, EMMeans pairwise comparisons).

Leafminer herbivory induces JA and ABA signalling pathways in the roots of wild-type plants but not in *def-1*

To determine which plant systemic signals mediate leafminer modulation of conspecific pupae development in the soil, we measured the concentrations of defence-related hormones (Fig. 2) and the expression levels of hormonal marker genes (Fig. 3) in the leaves and roots of noninfested and infested wild-type and *def-1* plants. Constitutive levels of OPDA and JA were overall higher in the leaves of *def-1* plants compared with the wild-type (ANOVAs, *genotype*, $P < 0.05$) (Fig. 2a), which was in line with previous reports (Howe *et al.*, 1996; Li *et al.*, 2002). Expression levels of the JA-associated gene marker *PI-IIf* were higher in wild-type leaves (ANOVA, *genotype*, $P < 0.001$) (Fig. 3a). This might be explained by a potential deficiency in OPDA metabolism and signalling, which controls the expression of proteinase inhibitors (Bosch *et al.*, 2014) in *def-1* plants (Howe *et al.*, 1996). Levels of SA did not differ between both genotypes (Fig. 2a). However, we observed a higher expression of *SAMT* in *def-1* leaves (ANOVA, *genotype*, $P < 0.001$) (Fig. 3a). This gene is responsible for the conversion of SA into methyl salicylate (MeSA) and therefore involved in the activation of systemic SA signalling. Upon leafminer infestation, SA levels were significantly reduced in both wild-type and *def-1* leaves (ANOVA, *herbivory*, $P < 0.001$) (Fig. 2a). Leaf levels of OPDA, JA, JA-Ile and ABA were not affected by the leafminer infestation. Accordingly, the expression of the JA-responsive gene marker *PI-IIf* did not differ between noninfested and infested plants (Fig. 3a). Leafminer infestation did not affect the expression of the SA-responsive gene marker *PR1a* either, but it significantly reduced the expression of the ethylene (ET)-responsive marker *ERF2A* (ANOVA, *herbivory*, $P < 0.001$) (Fig. 3a). This suppression was not accompanied by a lower expression of the ET-biosynthetic-related gene *ACO1*.

Levels of OPDA, JA and JA-Ile were higher in the roots of wild-type plants than in *def-1* (ANOVAs, *genotype*, $P < 0.05$). Aboveground leafminer infestation overall increased OPDA, JA-Ile and ABA levels in wild-type and *def-1* roots (ANOVAs, *herbivory*, $P < 0.05$) (Fig. 2b). When compared with their noninfested controls, however, differences in JA-Ile and ABA concentrations were slightly higher in the wild-type (JA-Ile, $P = 0.06$; ABA, $P < 0.05$; wild-type noninfested vs wild-type infested, EMMeans pairwise comparisons). *PI-IIf* expression levels were also higher in the roots of infested wild-type plants when compared with noninfested controls, and the opposite pattern was observed for *def-1* (ANOVA, *genotype* × *herbivory*, $P < 0.01$) (Fig. 3b). In addition to jasmonates, SA levels were also higher in the roots of leafminer-infested plants, albeit this was not statistically significant (ANOVA, *herbivory*, $P > 0.05$) (Fig. 2b). Relative expression levels of both the SA-responsive marker *PR1a* and SA-associated signalling *SAMT* genes, however, were significantly induced by leafminer attack in *def-1* only (ANOVA, *genotype* × *herbivory*, $P < 0.05$) (Fig. 3b). This suggests that there might be a stronger induction of SA signalling in the roots of the jasmonate-deficient tomato mutant upon leafminer infestation.

Modulation of pupal development is not dependent on the severity of leaf attack

The severity of plant damage upon herbivory can influence the magnitude of systemic plant responses, and, by consequence, plant–herbivore interactions (Robert *et al.*, 2012b). We therefore tested whether leaf damage by *L. trifolii* correlated with pupal development in the soil of wild-type and *def-1* plants. No significant difference in the number of leaf mines was found between wild-type and *def-1* plants (Fig. 4a; $t = 0.75$, $df = 17.86$, $P = 0.45$, Student's *t*-test). Similarly, no significant correlation between the

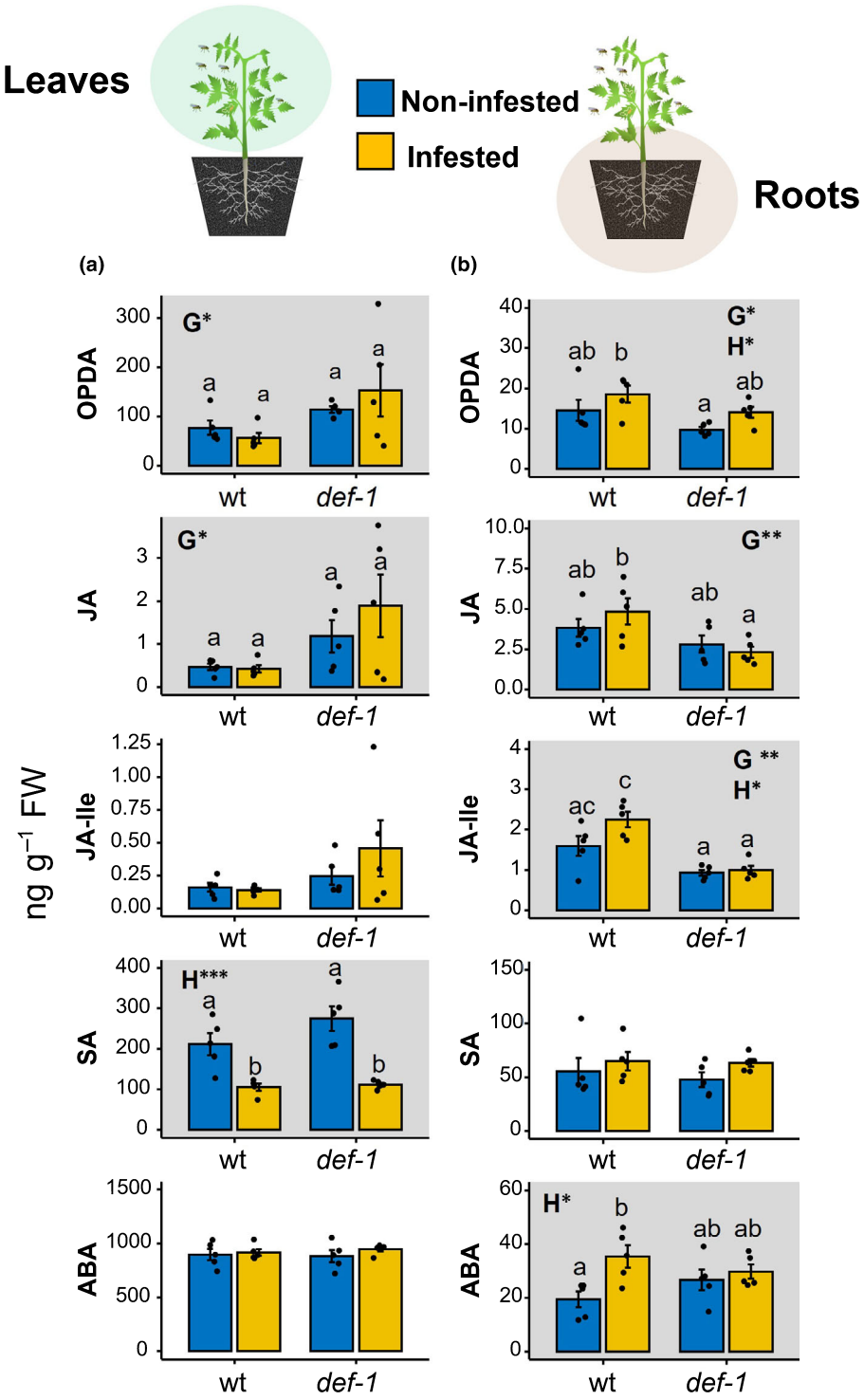

Fig. 2 Aboveground leafminer infestation triggers distinct, *def-1*-dependent hormonal responses in leaves and roots. Concentrations (mean ± SEM, $n = 5$) of 12-oxo-phytodienoic acid (OPDA), jasmonic acid (JA), jasmonic acid–isoleucine (JA-Ile), salicylic acid (SA), and abscisic acid (ABA) in leaves (a) and roots (b) of *Liriomyza trifolii-infested* and noninfested wild-type (wt) and *def-1* tomato (*Solanum lycopersicum*) plants at 24 d after infestation. Shaded graphs indicate statistically significant effects of plant *genotype* (G), *herbivory* (H) and/or their interaction (G × H) determined using two-way analysis of variance (ANOVA) (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Different letters within plots indicate significant differences among groups tested by estimated marginal means (EMMeans) *post hoc* test with multiple comparison adjustments (Tukey's honest significant difference (HSD)) ($P \leq 0.05$).

number of leaf mines and the number of emerging adults at 14 d (Fig. S2a; $r = -0.08$, $P = 0.72$, Pearson) nor at 16 d ($r = -0.092$, $P = 0.69$, Pearson) was observed.

Leafminer infestation changes soil humidity, but this does not explain modulation of pupal development

Soil humidity is an important factor affecting pupal survival and adult emergence rate (Hou *et al.*, 2006; Chen & Shelton, 2007; Wen *et al.*, 2016). In most cases, extremely wet or dry soils can hinder adult emergence. We therefore investigated whether *L. trifolii* leafminer attack changed soil humidity by sampling the upper layer of the soil, and whether these changes are associated with the modulation of pupal development. Previous assays have shown that this sampling method is representative of the moisture of the soil bulk where the plants are grown (Fig. S3). Leafminer attack significantly reduced soil humidity in wild-type, but not in *def-1* plants (Fig. 4b; *genotype*, $P = 0.308$, *herbivory*,

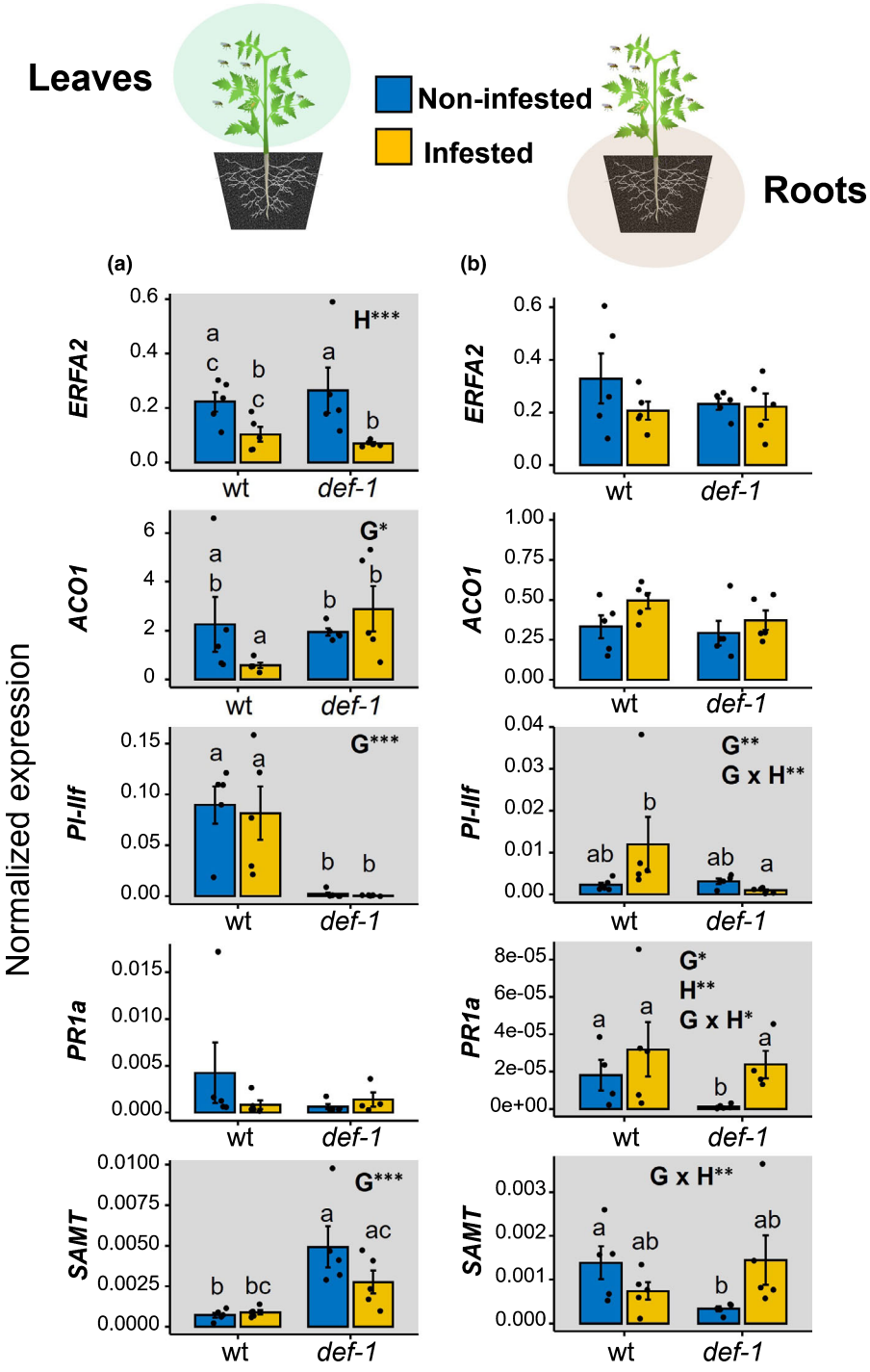

Fig. 3 Aboveground leafminer infestation modulates root defence gene expression in a *def-1*-dependent manner. Normalised transcript levels (mean ± SEM, $n = 4$ or 5) of marker genes for ethylene (*ERFA2* and *ACO1*), jasmonic acid (*PI-IIf*) and salicylic acid (*PR-1a* and *SAMT*) signalling in leaves (a) and roots (b) of *Liriomyza trifolii-infested* and noninfested wild-type (wt) and *def-1* tomato (*Solanum lycopersicum*) plants at 24 d after infestation. Transcript abundances were determined by quantitative reverse transcription polymerase chain reaction (qRT-PCR) and normalised to *Ubiquitin* transcript levels. Shaded graphs indicate statistically significant effects of plant *genotype* (G), *herbivory* (H) and/or their interaction (G × H) determined by two-way analysis of variance (ANOVA) (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Different letters within plots indicate significant differences among groups tested by estimated marginal means (EMMeans) *post hoc* test with multiple comparison adjustments (Tukey's honest significant difference (HSD)) ($P \leq 0.05$). Abbreviations for genes: *ACO1*, 1-aminocyclopropane-1-carboxylate oxidase 1; *ERFA2*, ethylene responsive factor A2; *PI-IIf*, *proteinase inhibitor IIf*; PR-1a, pathogenesis-related protein 1a; *SAMT*, salicylic acid carboxyl methyltransferase.

$P < 0.05$, *genotype* × *herbivory*, $P = 0.0511$, two-way ANOVA). Differences in soil humidity were not correlated with the number of emerging adults at 14 d (Fig. S2b; $r = -0.072$, $P = 0.66$, Spearman) nor at 16 d ($r = -0.12$, $P = 0.44$, Spearman).

Leafminer infestation modulates root volatile profiles in a *def-1*-dependent manner

Leaf herbivory can change root volatile production (Robert *et al.*, 2012a; Huang *et al.*, 2017), which may influence pupal development. To gain a first insight into the possible mechanism underlying the acceleration of leafminer pupae metamorphosis, we analysed root volatile content in infested and noninfested wild-type and *def-1* plants in a separate experiment. As leafminer infestation did not affect root biomass (Fig. S4; *genotype*, $P = 0.14$, *herbivory*, $P = 0.42$, *genotype* × *herbivory*, $P = 0.14$, two-way ANOVA), we expressed volatiles per g of root fresh weight. We detected 18 different volatile compounds in tomato roots using SPME-GC/MS. Redundancy analysis revealed that plant genotype, herbivory and their interaction explained 31.8% of the

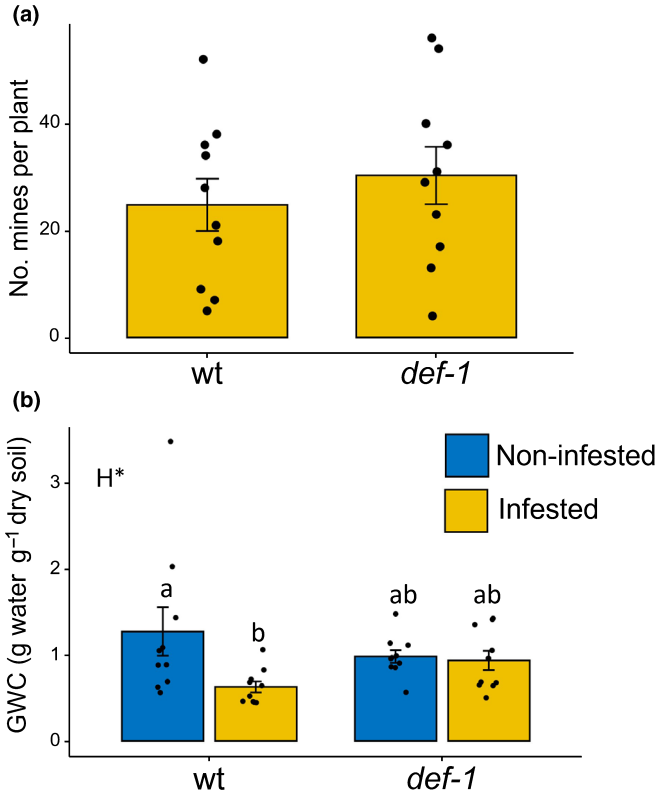

Fig. 4 Facilitation of leafminer pupae in the soil is not correlated with leaf damage and soil humidity. (a) Number of mines per plant (mean ± SEM, $n = 10$) determined in wild-type (wt) and *def-1* tomato (*Solanum lycopersicum*) plants at 24 d after *Liriomyza trifolii* infestation. Plants were individually infested with seven *L. trifolii* adult female flies allowing them to feed and lay eggs into the leaves of the plant. No differences were observed using Student's *t*-test at $P \leq 0.05$. (b) Soil humidity (mean ± SEM, $n = 10$) was estimated using the gravimetric water content (GWC) method in noninfested and *L. trifolii*-infested wt and *def-1* plants at 24 d after infestation. Effects of *herbivory* (H), plant *genotype* (G) and their interaction were tested using two-way analysis of variance (ANOVA). The statistically significant effects of herbivory are displayed in the graph (*, $P < 0.05$). Different letters indicate significant differences among groups tested by estimated marginal means (EMMeans) *post hoc* test with multiple comparison adjustments (Tukey's honest significant difference (HSD)) at $P \leq 0.05$.

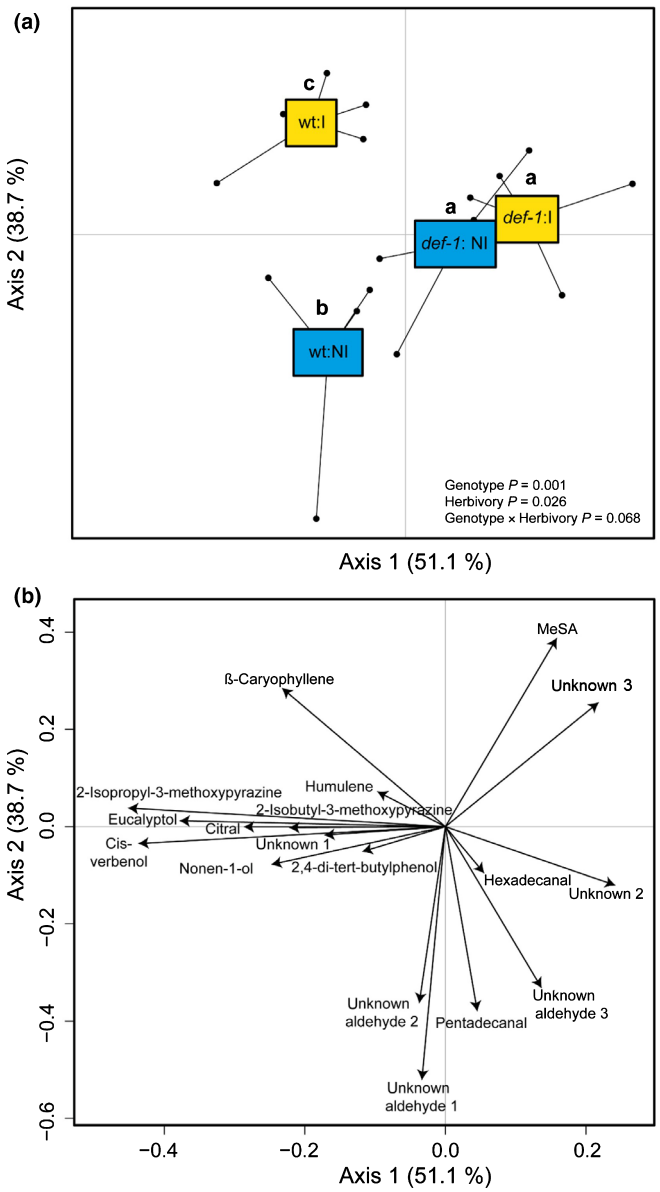

Fig. 5 Leafminer herbivory modulates root volatile profiles in a *def-1*-dependent manner. Redundancy analysis on root volatile profiles analysed by solid-phase micro-extraction–gas chromatography–mass spectrometry (SPME-GC-MS) in wild-type (wt) and *def-1* tomato (*Solanum lycopersicum*) plants at 24 d after *Liriomyza trifolii* infestation ($n = 5$). (a) Score plot showing the first two principal axis of the 'constrained PCA' with their explained variances in parentheses. Acronyms NI and I refer to noninfested and infested plants, respectively. The overall effects of plant genotype, herbivory and their interaction tested by permutation *F*-test are indicated. Different letters indicate significant differences among groups tested by permutation *F*-test pairwise comparisons corrected with the false discovery rate method at $P \leq 0.05$. (b) Loading plot displaying the contribution of each volatile compound on the separation of the treatments. Vector's length and direction denote the magnitude of their contribution and whether it is positive or negative, respectively.

total chemical variation (constraint variance) (Fig. 5; $F = 2.48$, $df = 3$, $P = 0.001$, permutation *F*-test on the canonical R^2). The remaining 68.2% of the variation (unconstraint variance) could be due to noise in the data, or unknown factors. In the associated score plot, the first axis accounted for 51.1% of the constraint variance explained by the variables and separated wild-type plants from *def-1* mutant plants (Fig. 5a). The second axis explained 38.7% of the constraint variance and separated *L. trifolii*-infested from noninfested wild-type plants (Fig. 5a), with infested and noninfested *def-1* mutant plants clustering together in the middle. Permutation *F*-tests on individual variables confirmed this pattern and revealed significant differences in volatile profiles between plant genotypes and between infested and noninfested wild-type plants, but not between infested and noninfested *def-1* plants (Fig. 5a).

Analysis of individual volatiles revealed that the levels of 1-8-cineole (Eucalyptol), 2-isopropyl-3-methoxypyrazine, 2-isobutyl-3-methoxypyrazine, *cis*-verbenol and 3,7-dimethyl-2,6-octadienal (Citral) were significantly higher in wild-type roots compared

with *def-1* roots (ANOVAs, *genotype*, $P < 0.05$) (Figs 5b, 6). Leafminer infestation slightly increased the release of MeSA in both genotypes (ANOVAs, *herbivory*, $P < 0.05$) (Fig. 6). Leafminer infestation suppressed the production of an unknown aldehyde (#1) in the roots of wild-type plants, but not *def-1* mutant plants (ANOVAs, *genotype* × *herbivory*, $P < 0.05$) (Fig. 6). A significant interaction between leaf infestation and genotype was also detected for (*E*)-β-caryophyllene (Fig. 6), which was induced by leaf infestation in the wild-type but not in *def-1* plants (although pairwise comparisons were not significant for this compound). Trends detected by SPME were confirmed for a subset of volatiles using a pentane extraction method followed by GC-MS analysis (Fig. S5). In particular, citral levels were significantly lower in *def-1* roots (ANOVA, *genotype*, $P < 0.01$), and induced by aboveground herbivory in a *def-1*-dependent manner (ANOVA, *genotype* × *herbivory*, $P < 0.05$). Eight additional compounds were detected in the pentane extracts of wild-type and *def-1* roots. The levels of some of these compounds differed between wild-type and *def-1* plants (ANOVA, *genotype*, $P < 0.05$), but none of them were significantly affected by herbivory.

Leafminer herbivory suppresses the expression of terpenoid biosynthetic genes in the leaves but not in the roots

To better understand how plant genotype and leafminer herbivory influence the production of root volatiles, we analysed the expression levels of terpene biosynthetic genes known to be involved in the production of the sesquiterpene β-caryophyllene and the monoterpene 1-8-cineole (Eucalyptol) (Fig. 7a) (Zhou & Pichersky, 2020) in the roots of noninfested and leafminer-infested plants. Levels of these compounds were significantly affected either by the plant genotype (Eucalyptol) or differently induced in the roots of infested wild-type plants (β-caryophyllene) (Fig. 6). To determine the biological significance of these terpene biosynthetic genes in leafminer–tomato interactions, we also analysed their expression levels in leaves. Constitutive levels of sesquiterpene-related (*FPPS1* and *TPS12*) and some of the monoterpene-related (*GGPPS3* and *CPT1*) biosynthetic genes were lower in the leaves of *def-1* plants compared with the wild-type (ANOVAs, *genotype*, $P < 0.05$) (Fig. 7b). This might be explained by the reduced density of type-VI glandular trichomes, where these compounds are produced and stored, reported for this mutant (Peiffer *et al.*, 2009; Escobar-Bravo *et al.*, 2017). Leafminer infestation suppressed *GGPS1* expression (involved in the production of precursors for monoterpene biosynthesis) in the leaves of both genotypes (ANOVA, *herbivory*, $P < 0.001$). The same pattern was observed for *GGPS3*, *CPT1* and *SSU1*, albeit only statistically significant for *SSU1* and with a stronger suppression effect in *def-1* (ANOVA, *genotype* × *herbivory*, $P < 0.01$). In the roots, *SSU1* was less expressed in *def-1* plants than in the wild-type (ANOVA, *genotype*, $P < 0.05$) (Fig. 7c), which mirrored the reduced levels of Eucalyptol detected in this genotype (Fig. 6). The expression pattern of *FPPS1* and *TPS12*, involved in β-caryophyllene biosynthesis, also mirrored the pattern of production of this compound in the roots of infested wild-type and *def-1* plants, but this was not statistically significant (Figs 6, 7c).

Discussion

Plants and herbivores interact dynamically with each other, with plants trying to mount appropriate defence responses and herbivores attempting to disrupt these responses and to modulate plant metabolism to their own benefit (Erb & Reymond, 2019). Here, we demonstrated that leafminer attack of aboveground plant tissues triggers systemic changes in the root signalling and metabolism that accelerate the development of the leafminer pupae in the soil adjacent to the plant. Below, we discuss this phenomenon from ecological and physiological points of view.

Over evolutionary time, herbivores have developed numerous strategies to feed and develop on well defended plants, including the systemic manipulation of plant metabolism to improve their own performance and fitness (Sarmento *et al.*, 2011; Robert *et al.*, 2012a; Schimmel *et al.*, 2017; Xu *et al.*, 2019). Leaf damage by the red milkweed beetle *Tetraopes tetraophthalmus*, for instance, increases larval survival on the roots of the same plants (Erwin *et al.*, 2014). Similarly, aboveground feeding by adult *Bikasha collaris* beetles on *Triadica sebifera* plants enhances the survival of conspecific larvae on the roots while reducing the performance of heterospecific herbivores (Huang *et al.*, 2013, 2014; Sun *et al.*, 2019). Whether insect herbivores can also modify plant metabolism to directly boost pupation – a critical step in their development – has not been investigated so far. The results reported here therefore represent a new mechanism by which herbivores can modulate the performance of their offspring. Developmental time is an important component of insect fitness, the latter defined as the total number of offspring produced by an individual during its lifetime that survive to join the mating population of the next generation (Thompson *et al.*, 2011). Fast life-history strategies including high growth rates and fecundity, at the cost of reduced longevity, allow the rapid increase in insect population sizes in seasonal habitats (Halali *et al.*, 2021). Faster pupal development may therefore increase *L. trifolii* performance by shortening its life cycle, therefore potentially allowing an increase in the number of generations per year. More importantly, faster pupal development may reduce the risk of attack by natural enemies (such as entomopathogens) in the soil (Liu *et al.*, 2009) by either shortening the exposure time and/or the period of vulnerability to biological control agents. Predation or infection success by natural enemies can be highly influenced by their insect host age and size (Nguyen *et al.*, 2007; Belliure *et al.*, 2008). For instance, increased growth rate of insect larvae can reduce their period of vulnerability to predation, as larger individuals are less attacked by predatory bugs (Belliure *et al.*, 2008). In addition, as the immune system also changes during the insect's development, it can influence the defence responses to parasitoids and diseases (Robb & Forbes, 2006; Eleftherianos *et al.*, 2008). Altogether, these mechanisms may promote outbreaks of pest species (Raymond *et al.*, 2002). However, it should be noted that a rapid development might entail physiological and ecological trade-offs as well. For instance, faster developmental

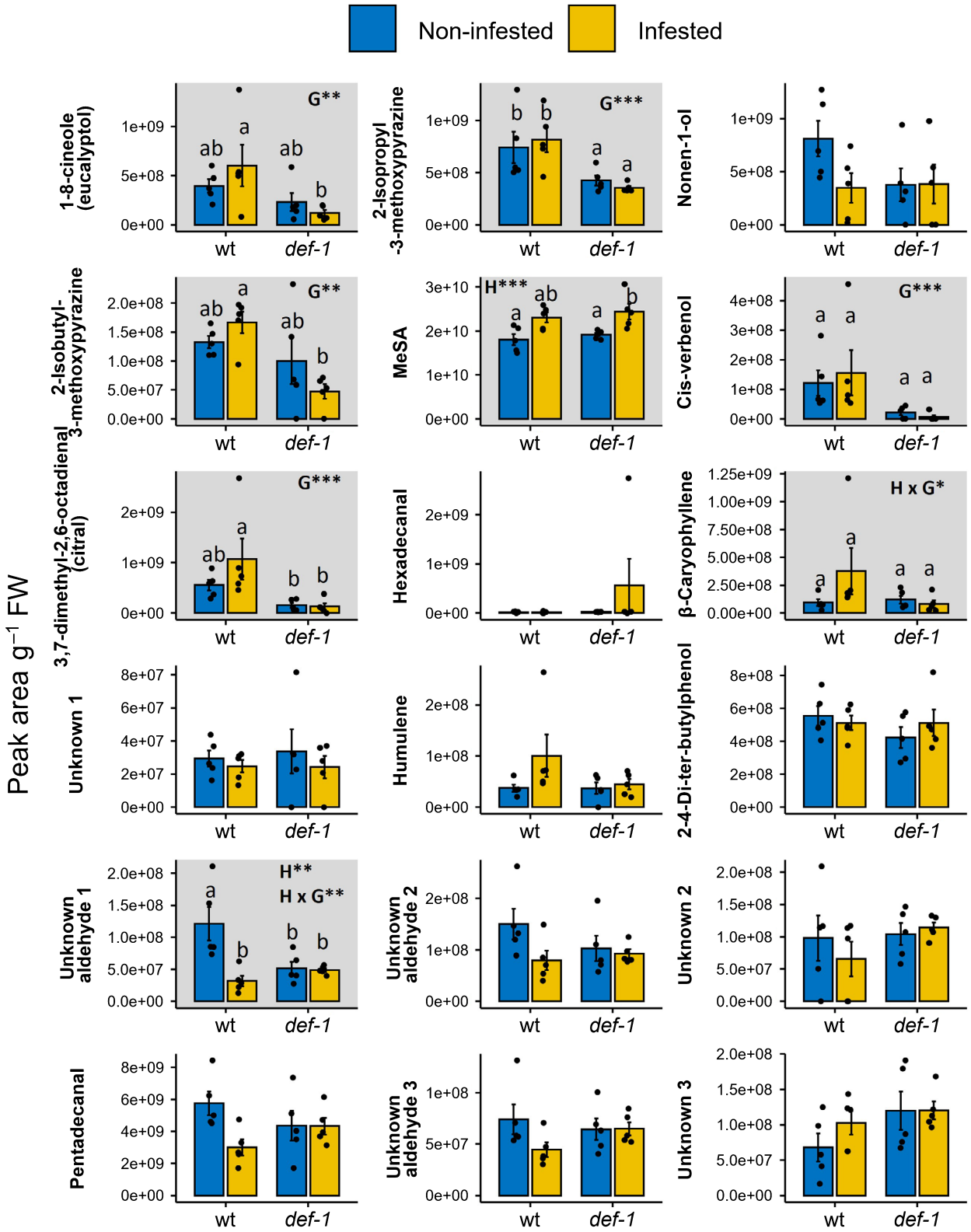

Fig. 6 Leafminer herbivory modulates the accumulation of individual volatile compounds in tomato roots. Levels of root volatiles (mean ± SEM, $n = 5$) detected in wild-type (wt) and *def-1* tomato (*Solanum lycopersicum*) plants at 24 d after *Liriomyza trifolii* infestation by solid-phase micro-extraction–gas chromatography–mass spectrometry (SPME-GC-MS). Shaded graphs indicate statistically significant effects of plant *genotype* (G), *herbivory* (H) and/or their interaction (G × H) determined by two-way analysis of variance (ANOVA) (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Different letters indicate significant differences among groups tested by estimated marginal means (EMMeans) *post hoc* test with multiple comparison adjustments (Tukey's honest significant difference (HSD)) ($P \leq 0.05$). MeSA, methyl salicylate.

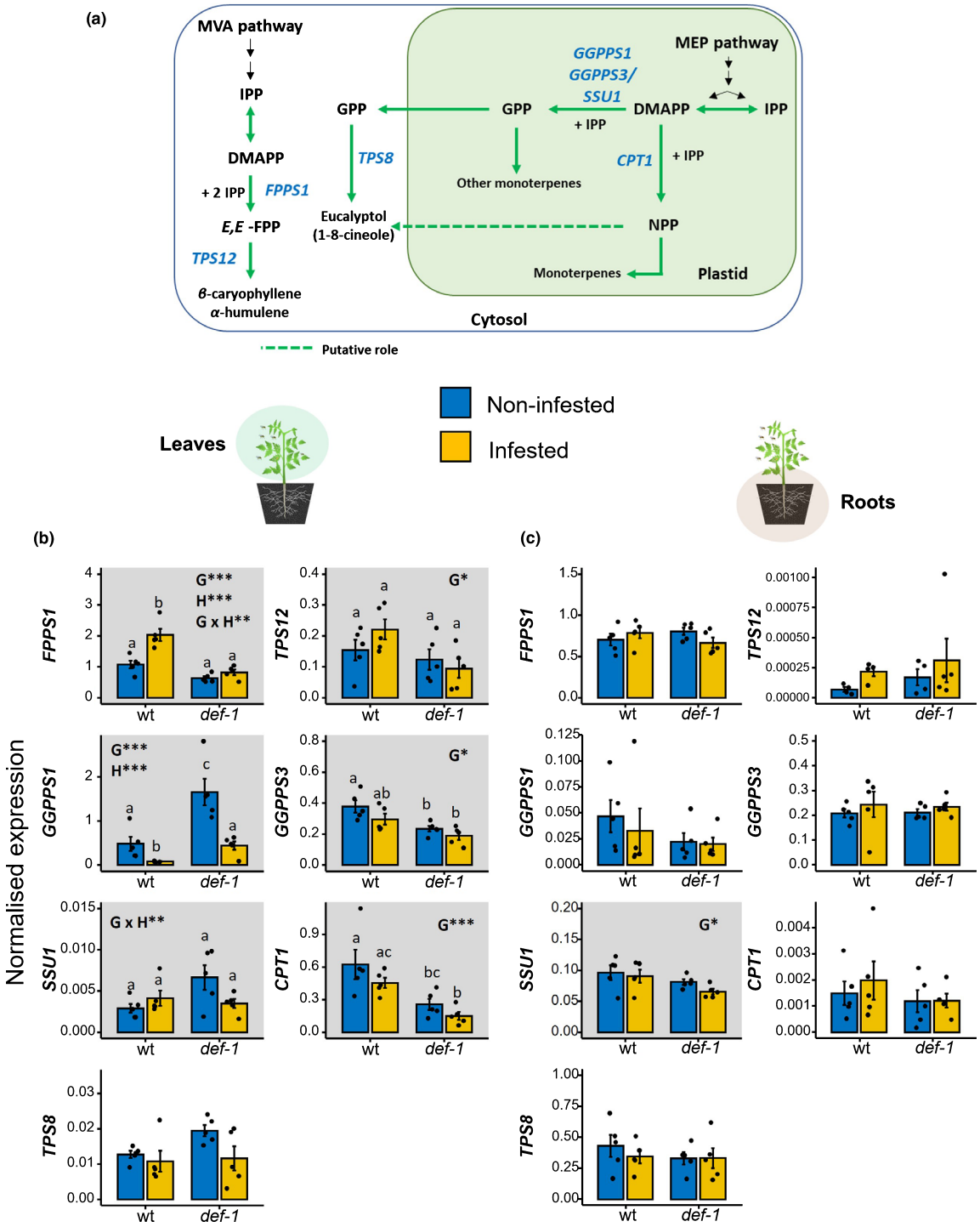

Fig. 7 Leafminer herbivory effects on the expression of terpene biosynthetic genes in leaves and roots. (a) Simplified scheme displaying the biosynthesis pathway of β-caryophyllene and Eucalyptol (1,8-cineole) in tomato (Zhou & Pichersky, 2020). Terpene precursors are synthesised by the cytosolic mevalonic acid (MVA) pathway and the plastidic methylerythritol phosphate (MEP) pathway. Normalised transcript levels (mean ± SEM, $n = 4$–5) of terpene metabolism-related genes in (b) leaves and (c) roots of *Liriomyza trifolii-infested* and noninfested wild-type (wt) and *def-1* tomato (*Solanum lycopersicum*) plants at 24 d after infestation. Transcript abundances were determined by quantitative reverse transcription polymerase chain reaction (qRT-PCR) and normalised to *Ubiquitin* transcript levels. Shaded graphs indicate statistically significant effects of plant *genotype* (G), *herbivory* (H) and/or their interaction (G × H) determined by two-way analysis of variance (ANOVA) (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Different letters indicate significant differences among groups determined by estimated marginal means (EMMeans) *post hoc* test with multiple comparison adjustments (Tukey's honest significant difference (HSD)) ($P \leq 0.05$). Abbreviations for precursors and genes: *CPT1*, cis-prenyltransferase 1; DMAPP, dimethylallyl diphosphate; *FPPS1*, farnesyl pyrophosphate synthase 1; *GGPPS1*, geranylgeranyl diphosphate synthase 1; *GGPPS3*, geranylgeranyl diphosphate synthase 3; GPP, geranyl diphosphate; IPP, isopentenyl diphosphate; NPP, neryl-diphosphate; *SSU1*, small subunit of geranyl diphosphate synthase 1; *TPS8*, terpene synthase 8; *TPS12*, terpene synthase 12. Dashed arrow indicates the putative route.

times in insects can result in reduced adult biomass (Halali *et al.*, 2021), which in some cases correlates with lower reproductive performance and fecundity (Awmack & Leather, 2002). In addition, an increased growth rate might potentiate intraspecific competition for the resources, which can negatively affect insect population growth (Agrawal, 2004). This aspect is especially relevant for *L. trifolii*, as larval performance can be negatively affected by an increasing number of mines per plant (Facknath, 2012). Further work will reveal the importance of this form of plant-mediated interaction for *L. trifolii* population dynamics and its interactions with other herbivore species and trophic levels. As many insect herbivores pupate on plants or in the soil close to plants, and are therefore exposed to plant chemicals, we expect plant-mediated effects of herbivory on pupation success to be common in nature.

Herbivores may modulate the performance of other plant-associated herbivore species or life stages through different mechanisms, including direct (Kunert *et al.*, 2005) and plant-mediated effects (Soler *et al.*, 2012). Here, we present several lines of evidence in support of the second scenario. First, the modulation of leafminer pupal development by aboveground feeding conspecifics depends on the tomato genotype, with acceleration occurring in wild-type plants and suppression in *def-1* mutants. *Def-1* is deficient in herbivory-induced JA accumulation (Li *et al.*, 2002) and defence induction, suggesting that the acceleration of pupal development observed in our study is jasmonate dependent. Indeed, our data show that aboveground attack by the leafminer induced JA and ABA signalling in the roots of wild-type plants but not in *def-1*. This finding is in line with the central role of jasmonates and ABA in systemic signalling between leaves and roots (Baldwin & Zhang, 1997; Erb *et al.*, 2011; Machado *et al.*, 2013). We also found that induction of SA signalling by leafminer infestation in *def-1* roots coincided with delayed pupal development in the soil, suggesting a possible molecular mechanism for the reverse pattern in the tomato mutant. Second, leafminer attack triggers changes in soil humidity and root volatile profiles in a *def-1*-dependent manner, which is consistent with the notion that leaf attack modulates the physiology and metabolism of tomato roots in a way that accelerates pupal development in the soil. Finally, it should be kept in mind that leafminers, in contrast with other chewing herbivores, essentially live within the leaves. This means that they are unlikely to release high quantities of body odours and frass into the soil environment and influence their conspecific pupae (Hering, 1951). Taken together, these results strongly suggest that leafminers accelerate pupal development by inducing systemic changes in their host plant.

What is the eco-physiological mechanism by which tomato roots of leafminer-infested plants accelerate pupal development in the soil? As the pupae in our experiments did not have direct contact with tomato roots and their exudates, changes in root structure, endogenous root chemistry and nonvolatile exudates can be excluded. Conversely, soil-dwelling pupae are known to be highly sensitive to changes in soil humidity (Benoit *et al.*, 2010; Barnett & Johnson, 2013), and they may also be influenced by root volatiles, which are known to modulate root herbivore behaviour and performance (Rasmann *et al.*, 2005; Ali *et al.*, 2011; Robert *et al.*, 2012a,b; Huang *et al.*, 2017). We found that aboveground leafminer infestation significantly reduced soil humidity in wild-type plants, but not in *def-1* plants. Correlation analyses, however, did not show any association between soil humidity and pupal developmental speed. Furthermore, pupal development was slower in leafminer-infested than noninfested *def-1* mutants, even though soil humidity was not significantly affected by leafminer attack in this genotype. Alternatively, the fact that the leafminer pupae were not directly in contact with the soil, but instead placed inside cages, might have minimised the potential effects of variations in soil moisture. Therefore, our data do not support a role of soil humidity in the systemic modulation of pupation time. Instead, we infer that changes in belowground volatiles, either plant or soil derived, may be driving the observed patterns. In line with this, we found that the volatile profiles of control (noninfested) wild-type and *def-1* roots significantly differed, which could account for the observed differences in the leafminer pupae developmental times between both genotypes. Upon leafminer attack, however, we observed significant changes in the volatile profiles of wild-type roots but not in the roots of the tomato mutant *def-1*. Individual analyses of the root volatile compounds showed that leafminer herbivory reduced the levels of an unknown aldehyde (#1) and slightly enhanced the accumulation of (*E*)-β-caryophyllene and citral in wild-type roots, but not in *def-1*. Although we did not find any volatiles that show a statistically significant inversion of responsiveness between the genotypes, tendencies for this phenomenon were also found to occur for Eucalyptol and a isobutyl methoxypyrazine, two well known plant aroma compounds (Lamy *et al.*, 2017; Murungi *et al.*, 2018), with Eucalyptol having antimicrobial properties (Kifer *et al.*, 2016). As belowground pupae are susceptible to fungal diseases, the differential emission of root volatiles may modulate the microbial load of the pupae, thereby indirectly altering their development. In addition, other belowground volatiles that were not detected in our analyses may have affected pupal development. For instance, belowground ethylene emissions can be modulated by aboveground herbivory (Robert *et al.*, 2012a), making this compound a target for future analyses. In summary, both *def-1*-dependent and herbivory-induced changes in root chemical profiles are likely to account for the observed differences between *def-1* and wild-type plants, but further research is needed to determine whether their effects on leafminer pupal development are mediated by the same or different belowground compound/s.

Our attempt to disentangle how leafminers attack affects the root chemistry at the molecular level showed that expression of terpene biosynthetic genes involved in the production of sesquiterpenes and monoterpenes were overall suppressed in the leaves of wild-type and *def-1* plants, but not affected in the roots. However, some genes involved in Eucalyptol biosynthesis mirrored the induced levels of this compound in the wild-type upon leafminer infestation. Herbivore-mediated induction of terpene biosynthesis is a dynamic process, with expression of biosynthetic genes oscillating after herbivory events and diurnal cycles, and often temporally uncoupled from the terpene emission

(Seidl-Adams *et al.*, 2015). Most of the studies determining the kinetics of this induction have focused on early hours after herbivory, but the kinetics of this biosynthetic pathway in long-term herbivore-infested plants are largely unknown. Furthermore, terpene biosynthesis in the roots might display its own regulatory mechanism, differing from those described in the leaves. Because wild-type and *def-1* plants were continuously exposed to leaf-feeding leafminers during the experiments, a more detailed time-course analysis covering different infestation times will shed light on the herbivory-mediated regulation of this metabolic pathway in the roots as well as the responses of leafminer pupae to different exposure times of root volatiles. Taken together, our work shows that systemic changes in tomato roots trigger belowground volatile-mediated changes in pupal development following leafminer attack. Future experiments should address the role of root volatiles relative to volatile compounds that are produced by rhizosphere and soil microbes. Disentangling these factors will be challenging, as they would require microbe-free plants, substrates and insect pupae, but experiments with synthetic volatiles could unravel their specific impact on pupal development in the soil.

In conclusion, our study adds a novel facet to plant–herbivore interactions by demonstrating that leaf herbivory can modulate pupal development in the soil via plant-mediated changes in belowground volatiles. This finding represents a first step towards unravelling the molecular mechanisms and ecological significance of herbivory-induced modulation of insect metamorphosis.

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Author contributions

RE-B conceived the study. RE-B and ME designed experiments. RE-B and BCJS performed experiments. RE-B and GG performed the hormone analyses. RE-B and BCJS performed gene expression analyses. RE-B analysed the data. ME and PGLK supervised the study. RE-B, BCJS, ME and PGLK interpreted the data. RE-B wrote the first draft of the manuscript and all authors contributed to revisions.

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Data availability

The data generated for this manuscript have been archived in the Dryad Digital Repository, and it is available under the following doi: 10.5061/dryad.sj3tx9669.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Pictures of the experimental set-up used to test the effect of belowground volatiles on leafminer pupal development.

Fig. S2 Correlation analyses between the cumulative number of emerged adults and the number of mines per plant and the soil moisture.

Fig. S3 Correlation analysis between the soil moisture determined at different soil strata and from different soil volumes.

Fig. S4 Root dry biomass determined in noninfested and leafminer-infested wild-type and *def-1* plants.

Fig. S5 Levels of tomato root volatiles determined in pentane extracts of noninfested and *Liriomyza trifolii*-infested wild-type and *def-1* plants.

Table S1 qRT-PCR primer specifications.

Table S2 Estimated marginal means, standard errors and confidence limits.

Table S3 *P*-values associated with pairwise comparisons of estimated marginal means.

Table S4 Analysis of deviance table (type II Wald chi-squared tests).

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