Title: Maize integrates light and volatile cues from neighboring plants into stronger volatile emissions

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Supporting Information: Figs. S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11 and S12, Table S1, and Methods S1

Abstract

- Plants perceive the presence and defense status of their neighbors through light and volatile cues, but how plants integrate both stimuli is poorly understood.
- We investigated if and how low Red to Far red light (R:FR) ratios, indicative of shading or canopy closure, affects maize (*Zea mays*) responses to herbivore-induced plant volatiles (HIPVs), including the green leaf volatile (*Z*)-3-hexenyl acetate. We modulated light signaling and perception by using FR supplementation and a *phyB1phyB2* mutant and we determined volatile release as response readout. To gain mechanistic insights, we examined expression of volatile biosynthesis genes, hormone accumulation, and photosynthesis.
- Exposure to a full blend of HIPVs or (*Z*)-3-hexenyl acetate induced maize volatile release. Short-term FR supplementation increased this response. In contrast, prolonged FR supplementation or constitutive phytochrome B inactivation in *phyB1phyB2* plants showed the opposite response. Short-term FR supplementation enhanced photosynthesis and stomatal conductance, and (*Z*)-3-hexenyl acetate-induced JA-Ile levels.
- We conclude that FR-enriched light environments can prompt maize plants to respond more strongly to HIPVs emitted by neighbors, which might be explained by changes in photosynthetic processes and phytochrome B signaling. Our findings reveal interactive responses to light and volatiles cues with potentially important consequences for plant-plant and plant-herbivore interactions.

Key words

Herbivore, far-red light, green leaf volatiles, maize, phytochrome B, plant defenses, signal integration, terpenes

Introduction

The capacity to perceive and respond to fluctuating environments is essential to life on earth. As primary producers in terrestrial ecosystems, plants are constantly dealing with competitors, pests, and pathogens. Consequently, they have evolved systems to detect these stressors and respond to them appropriately. While recent work has advanced our knowledge of how plants perceive individual environmental cues to detect biotic stressors, much less is known about their capacity to simultaneouly integrate multiple ones (Nguyen *et al.*, 2016; Escobar-Bravo *et al.*, 2017).

Plants can perceive qualitative and quantitative changes in light. When plants grow in dense canopies there is a strong decline in photosynthetically active radiation (PAR) and a change in light quality due to the light absorption and reflection by leaves (Ballaré & Pierik, 2017). Plants typically absorb red (R) and blue (B) light whereas the far red (FR) component of the sunlight is mostly reflected and transmitted. Consequently, in dense or closing canopies, there is a decrease in the R to FR ratio (R:FR) resulting from the reduction in R light, which is strongly absorbed by chlorophylls, and the increase in FR radiation. A reduction in R:FR ratio is a signal of foliar shading and proximity of other plants (Ballaré *et al.*, 1990). Plants can respond to this FR-enriched light environment by upward bending of the leaves (hyponasty) and stem elongation, which allows them to reach more illuminated areas and outcompete their neighbors (Franklin, 2008; Ballaré & Pierik, 2017). Variations in R:FR ratios are perceived by the photoreceptor phytochrome B (phyB) (Fankhauser, 2001). PhyB is inactivated under low R:FR, which triggers the expression of growth-related pathways controlled by hormones such as auxins and gibberellins (Fiorucci & Fankhauser, 2017).

Besides light cues, plants can perceive and respond to volatile organic compounds emitted by herbivores (Helms *et al.*, 2017) and neighboring plants (Hu, 2022). When a plant is attacked, it releases distinct blends of herbivore induced plant volatiles (HIPVs). HIPVs can be perceived by non-attacked plants and induce or prime their defenses (Engelberth *et al.*, 2004). Plant responses to HIPVs includes the rapid release of volatiles (Engelberth *et al.*, 2004) and HIPV-induced priming, which allows plants to respond more rapidly and strongly once they are under attack (Ton *et al.*, 2007). In maize, HIPVs such as indole and (*Z*)-3-hexenyl acetate [(*Z*)-3-HAC] prime the jasmonic acid (JA) pathway (Erb *et al.*, 2015; Ye *et al.*, 2019), triggering the deployment of an array of defenses that enhance herbivore resistance and the attraction of parasitoids (Ton *et al.*, 2007). In addition, (*Z*)-3-HAC can directly induce defenses, volatile biosynthesis, and release (Engelberth *et al.*, 2004; Hu *et al.*, 2019).

Recent work has demonstrated that R:FR ratios influence defense deployment in plants, including volatiles. Reduced R:FR via FR supplementation to ambient light suppresses direct defenses against herbivores and pathogens (Moreno *et al.*, 2009; Cargnel *et al.*, 2014; Courbier *et al.*, 2021). This is predominantly mediated by crosstalk between phyB and the JA pathway (Moreno *et al.*, 2009; Campos *et al.*, 2016; Fernández-Milmanda *et al.*, 2020). When phyB is inactivated, JA signaling is

attenuated as a consequence of reduced levels of bioactive JA forms (Fernández-Milmanda *et al.*, 2020), decreased stability of MYC transcription factors (Chico *et al.*, 2014), and enhanced stability of JAZ transcriptional suppressors (Chico *et al.*, 2014; Leone *et al.*, 2014). As volatile biosynthesis can be regulated by JA, this might explain why phyB inactivation by low R:FR ratios modulates the emission of some volatile compounds. Upon FR supplementation, for instance, barley (*Hordeum vulgare*) plants show reduced constitutive emission of several terpenes but an increased emission of the monoterpene linalool oxide (Kegge *et al.*, 2015). Similarly, reduced R:FR via FR supplementation in combination with shading (i.e., low PAR) reduces constitutive and methyl jasmonate-induced terpenes and green leaf volatiles emissions in *Arabidopsis thaliana*, but induces the emission of ethylene, a volatile phytohormone commonly deployed upon low R:FR (Kegge *et al.*, 2013). The fact that emission of specific volatiles is enhanced under low R:FR (Kegge *et al.*, 2015; Cortés *et al.*, 2016) suggests that the response to competition signals may be highly specific.

How does the perception of light cues modulate the perception of volatiles emitted by neighbors? In contrast to the well documented effects of light cues on volatile emissions, close to nothing is known about this important question. That plants are capable of sensing and integrating different types of information is illustrated, for instance, by seed germination initiation, which requires a combination of environmental cues such as temperature, moisture, and light (Finch-Savage & Leubner-Metzger, 2006). Furthermore, plants that are under attack by herbivores of different feeding guilds often show unique response patterns due to the hormonal integration of different signals (Soler *et al.*, 2013; Zhang *et al.*, 2013; Kroes *et al.*, 2015; Chrétien *et al.*, 2018). Thus, plants might also integrate light and volatiles cues into different responses. For instance, plants might reduce their response to volatile cues when they perceive light cues that indicate the close proximity of neighboring plants as it may reduce the risk of an individual plant being attacked (Barbosa *et al.*, 2009).

In this study, we employed a newly developed automated high-throughput volatile screening platform with a programmable multi-channel LED lighting to investigate if and how maize (*Zea mays*) plants integrate light and volatile cues into defense-associated responses. We exposed maize plants to short-term FR supplementation as a light cue for vegetation shade and to HIPVs or (*Z*)-3-HAC as volatile cues for the presence of herbivore-infested neighbors, and we determined direct induction and priming responses of volatile emissions in time-series analyses. In addition, we used a maize $phyB_1phyB_2$ double mutant and short- or long-term FR supplementation experiments to determine the role of PhyB in maize integration of both environmental cues. To gain mechanistic insights, we determined gene expression, phytohormone levels, and photosynthetic responses in response to short-term FR treatments and (*Z*)-3-HAC. Our experiments demonstrate that short-term exposure to FR-enriched light environments, characteristic of early neighbors' detection, enhances maize volatile emissions in response to HIPVs and (*Z*)-3-HAC, illustrating how plants use different types of signals from neighbors to reprogram their own phenotypes.

Materials and Methods

Plants

Experiments were conducted with the maize (*Zea mays*) inbred line B73, the *phyB$_1$phyB$_2$* maize double mutant, and its wild-type W22. The *phyB$_1$phyB$_2$* double mutant was provided by Prof. Michael Scanlon (School of Integrative Plant Science, Cornell University). This mutant is homozygous for the Mutator (Mu) transposon insertion alleles *phyb1-563* and *phyb2-12058* resulting in no detectable PHYB protein (Sheehan *et al.*, 2007; Dubois *et al.*, 2010). B73 responds to (*Z*)-3-HAC (Hu *et al*., 2018) and FR (Dubois *et al*., 2010) and releases moderate volatile emissions upon herbivory (Block *et al*., 2018). For experiments with 11-day-old plants, seeds were sown in transparent cylindric plastic pots (11x4 cm) filled with commercial soil (Selmaterra, BiglerSamen, Switzerland) and wrapped with aluminum foil to prevent root exposure to light. For experiments with 30-day-old plants, seeds were sown in black plastic pots (9x9x10 cm) filled with the same commercial soil. Plants were grown in a ventilated greenhouse supplemented with artificial lighting under 50-70% relative humidity, 14/10 h light/dark photoperiod, and 14-22/10-14°C day/night temperatures.

Insects

Eggs of *Spodoptera littoralis* were provided by Oliver Kindler (Syngenta, Stein, CHE) and the larvae were reared on artificial diet as described in Maag *et al.*, 2014. *S. littoralis* oral secretions were collected from third- to fourth-instar larvae as described in Hu *et al.*, 2019 and stored at − 80°C until use.

Light treatments

Light treatments were conducted in a custom-made high-throughput phenotyping platform designed for volatile analyses. The platform consisted of 96 transparent cylindrical glass chambers (120 mm Ø, 450 mm height; abon life sciences, Switzerland) closed at the top with transparent glass lids and distributed over two tables. Each table was supplied with four light emitting diodes (LEDs) lamps placed above the glass chambers. Each lamp contained 120 LEDs with nine wavebands consisting of 380, 400, 420, 450, 520, 630, 660, 735 nm and 5700K white (RX30, Heliospectra). Individual glass chambers were supplied with an airflow of 0.8 L/min and an air outlet. Plants were placed inside these glass chambers and exposed to low (~0.5) or high (~2.65) R:FR ratios (R, λ = 600-700 nm; FR, λ = 700-800 nm) by providing supplemental FR to the same white light background (120 ± 15 µmol m^{-2} s^{-1}) (Supplemental Fig. S1) with a photoperiod of 16/8 h light/dark. This low R:FR corresponds to the ratios observed in high density maize canopies (Maddonni *et al.*, 2002), where low PAR levels as the ones used in our experimental set-up are commonly observed (Xue *et al.*, 2016). Light treatments were separated by white opaque curtains and started between 9:00 and 10:00 a.m. two days before the volatile exposure treatments.

(Z)-3-HAC treatment

Individual plants were exposed to one (*Z*)-3-HAC dispenser for 6 h within the transparent cylindrical glass chambers of the custom-made high-throughput phenotyping platform described above. (*Z*)-3-HAC dispensers were prepared as described in Hu *et al.*, 2019, each releasing approximately 70 ng h^{-1} , which has been previously described in herbivore-attacked maize plants (Mérey *et al.*, 2011). (*Z*)-3-HAC treatments started between 9:00-10:00 a.m.

Simulated herbivory

Two leaves (leaf 2 and 3 from the bottom) per plant were wounded over an area of ca. 0.5 cm^2 parallel to the central vein using a haemostat followed by the application of 10 µL of *S. littoralis* larval oral secretions (diluted 1:1 in autoclaved distilled water) in each wounding site. This treatment induces plant defense responses comparable to real herbivory (Erb *et al.*, 2009).

Linalool dispensers

Linalool dispensers were prepared as described in Hu *et al.*, 2019. The dispensers contained 100 µL of pure linalool (SIGMA) and were pierced through the rubber septum with a 1 µL micropipette (Drummond, Millan SA, Switzerland).

PTR-TOF-MS volatile analysis

Plant volatile emission was determined using the automated high-throughput phenotyping platform described above for light treatments. Volatiles emitted by a plant inside each transparent glass chamber were purged by a clean air flow (0.8 L/min) that carried the emitted volatiles directly to a Vocus proton transfer reaction time-of-flight mass spectrometer (PTR-TOF-MS) system (Tofwerk, Switzerland) for analysis. The Vocus PTR-TOF-MS was operated using H_3O^+ as the reagent ion and was integrated into an autosampler (Gonin *et al*., 2022) that cycles between individual chambers/plants. Each plant was measured for 50 or 60 seconds. Between individual plants, a fast zero air measurement was performed for 5 seconds. Complete mass spectra (0-500 Th) were saved and plotted at 5 Hz (5 unique mass spectra per second). PTR-TOF-MS data processing consisted of mass calibration and calculation of peak intensities using the Tofware software package v3.2.0, followed by normalization of the signals to aboveground (stem and leaves) plant fresh biomass.

GC-MS volatile emission analysis

Dynamic volatile collection was conducted using adsorbent Super-Q traps followed by gas chromatography-mass spectrometry (GC-MS) analysis of volatile extracts**.** For this, air was pulled out from the PTR-TOF-MS transparent glass chambers containing the test plants through the air outlet ports (0.5 L/min) and across the Super-Q traps (Porapak-Q™ 50 mg, Volatile collection Trap, USA). Volatiles were collected for periods of 2 h for 12 h. After each collection period, Super-Q traps were

exchanged for new ones and the volatiles collected on the trapping filters were extracted with 200 µl of Dichloromethane (Roth) containing 200 ng of both N-octane and nonyl-acetate as internal standards. Extracts were analyzed by GC-MS (Agilent 7820A GC interfaced with and Agilent 5977E MSD, Palo Alto, CA, USA). Aliquots of 1 µL were injected into the column (HP5-MS, 30 m, 250 µm ID, 2.5-µm film, Agilent Technologies, Palo Alto, CA, USA) using a pulsed split-less mode and He as carrier gas at a flow rate of 1 mL min^{-1}. A temperature gradient of 5°C min^{-1} from 40°C to 200°C was used. Compound identification was based on similarity to library matches (NIST search 2.2 Mass Spectral Library. Gaithersburg, MD, USA), retention times, and spectral comparison with pure compounds. Relative abundance was calculated by dividing the peak area of individual compounds by the aboveground plant fresh biomass.

Phytohormone analysis

The concentrations of 12-oxo-phytodienoic acid (OPDA), JA, jasmonic acid-isoleucine (JA-Ile), salicylic acid (SA), abscisic acid (ABA) and auxin (indole acetic acid, IAA) were determined in aboveground tissues (stem and leaves) following the procedure described in Glauser *et al.*, 2014 with minor modifications (Supplemental Methods S1).

Gene expression analysis

Total RNA was isolated using TRI Reagent (Sigma-Aldrich) following the manufacturer's instructions. DNase treatment, reverse transcription, and first strand cDNA synthesis were performed using gDNA Eraser (Perfect Real Time) and PrimeScript Reverse Transcriptase following manufacturer's instructions (Takara Bio Inc. Kusatsu, Japan). For gene expression analysis, 2 µL of cDNA solution (equivalent to 10 ng of total RNA) served as template in a 10 µL qRT-PCR reaction using ORA™ SEE qPCR Mix (Axonlab) on an Applied Biosystems® QuantStudio® 5 Real-Time PCR System. Normalized expression (NE) values were calculated as in Alba *et al.*, (2015). Gene identifiers, primer sequences, and references are listed in Table S1.

Stomatal conductance, density, and net photosynthesis

Stomatal conductance was measured using an AP4 Porometer (Delta-T Devices Ltd) with cup aperture dimension of 2.5 x 17.5 mm. Stomatal density was determined in epidermal imprints of the abaxial surface using clear nail varnish. Epidermal imprints were imaged using a Leica DM2500 optical microscope coupled to a Leika HD Digital microscope camera (Leica MC170 HD) (Leica Microsystems, Wetzlar, Germany). The number of stomata was determined in 10 pictures per plant and leaf using ImageJ software. Net photosynthesis was determined using a LI-6400 Portable Photosynthesis system (LI-COR, Biosciences).

Statistical analysis

Data analysis was conducted in R version 4.0.4 (R core Team, 2016). Effects of (1) sampling time, (2) light, (3) genotype, (4) volatile exposure, and (5) their interactions on volatile emissions in the different experiments were tested in linear mixed-effects models (LME) using nlme library. We included individual plants as a random factor and a correlation structure when autocorrelation among residuals was found to be significant ($p < 0.05$) in the models. Fitted models were subjected to type III analyses of variance (ANOVAs) to produce a summary of the F- and p statistics (car package Fox & Weisberg, 2018). When needed, data from volatiles signatures were log10 or squared root transformed prior analysis to correct for heteroscedasticity. In Fig. 4 differences among light treatments were tested for all volatiles at each sampling time by estimated marginal means. Hormone concentrations and gene expression were analyzed separately for each time point by two-way ANOVAs followed by Tukey HSD post hoc test when light, (*Z*)-3-HAC, or their interaction were significant. Effects of light, (*Z*)-3-HAC, herbivory, and their interactions on stomatal conductance, density, and net photosynthesis were tested by two-way ANOVAs followed by Tukey HSD post hoc test.

Results

FR-enrichment boosts maize responses to herbivore-induced plant volatiles

HIPVs can induce the emission of volatiles in non-attacked plants and enhance their defense responses when actual herbivory occurs (Farag & Paré, 2002; Engelberth *et al.*, 2004). To determine whether different R:FR ratios alter growth and responses to HIPVs in maize, we exposed 11-day old B73 seedlings to high or low R:FR for two days by modulating FR levels. Thereafter, we measured growth-related parameters and compared their volatile emissions when exposed to volatiles emitted by non-infested or *Spodoptera littoralis*-infested low R:FR treated maize plants. Low R:FR-treated emitter plants infested with ten second-instar *S. littoralis* larvae or left uninfested were connected to the glass chambers of receiver plants using Teflon tubing (Fig. 1a). The air flow moved from the emitter's chamber towards the receiver's chamber. Volatiles simultaneously emitted by emitter and receiver plants were collected from the glass chamber containing the receiver plant through an air outlet connected to the PTR-TOF-MS system. Because all emitter plants were exposed to low R.FR and volatile fluxes were unidirectional, differences in volatile emissions could only be explained by the light treatment of the receivers (low or high R:FR).

Low R:FR increased the elongation of stem internodes and auxin levels (Supplemental Fig. S2ab), confirming the effectiveness of our light treatment in triggering shade avoidance (Dubois & Brutnell, 2009).

Emission of the green leaf volatiles (GLVs) hexenal, hexen-1-ol and hexenyl acetate, as well as indole, monoterpenes, sesquiterpenes, and the homoterpenes DMNT and TMTT by *S. littoralis*-

infested emitter + intact receiver plants significantly increased over time in comparison to non-infested emitter + intact receiver plants (Fig. 1b). Indole and sesquiterpenes emissions were significantly higher when receiver plants were exposed to low R:FR and volatiles from *S. littoralis*-infested emitter plants than to the same volatiles and high R:FR (Fig. 1b). A similar pattern was observed for monoterpenes and DMNT. As emitter plants were all grown under low R:FR, showed similar levels of herbivore damage (Supplemental Fig. S3), and did not receive any volatile cues from the receiver plants, these differences must be the result of changes in the receivers' volatile emissions. Thus, FR-enriched light environments enhance maize responses to HIPVs emitted by herbivore-infested neighbors.

FR-enrichment boosts maize responses to (Z)-3-HAC

GLVs such as (*Z*)-3-hexenal, (*Z*)-3-hexen-1-ol, and (*Z*)-3-HAC are HIPVs emitted by maize that can induce the emission of volatiles in neighboring intact maize plants (Farag & Paré, 2002; Engelberth *et al.*, 2004). We next tested whether low R:FR modulates maize responses to the GLV (*Z*)-3-HAC. We exposed 11-day old B73 maize seedlings to low or high R:FR for two days by modulating FR levels and determined their volatile emissions by using the PTR-TOF-MS system during (*Z*)-3-HAC exposure and subsequent simulated herbivory (Fig. 2a). These analyses focused on indole and terpenoid emissions because these compounds responded to low R:FR and HIPVs in the previous experiment.

Exposure to (*Z*)-3-HAC induced volatile release in both high and low R:FR-treated maize plants (Fig. 2b). Low R:FR, however, significantly enhanced (*Z*)-3-HAC-induced emissions of monoterpenes and DMNT, while the opposite was observed for indole.

Upon simulated herbivory, pre-exposure to (*Z*)-3-HAC increased the emission of all the volatiles signatures in both light treatments (Fig. 2b). Emission of DMNT was significantly higher in low R:FR-treated plants than in high R:FR-treated plants pre-exposed to (*Z*)-3-HAC. Irrespective of (*Z*)-3-HAC pre-treatment, herbivory-induced plants exposed to low R:FR emitted more monoterpenes, sesquiterpenes, and DMNT than high R:FR-treated plants (Fig. 2b). An additional experiment confirmed that low R:FR enhances herbivore-induced emissions of monoterpenes and DMNT, but it reduced constitutive indole emissions and significantly increased them upon herbivory (Supplemental Fig. S4).

The PTR-TOF-MS system only detects molecular mass features, while the structural identity of isomers, e.g., different mono- or sesqui-terpenes, cannot be resolved. To determine the identity of the induced volatile compounds and confirm the observed patterns with an established, orthogonal method, we collected plant volatiles for periods of 2 h for 12 h using Super-Q traps followed by GC-MS analysis of volatile extracts. (*Z*)-3-HAC exposure significantly increased the emissions of indole, the monoterpene alcohol linalool, the sesquiterpenes (*E*)-β-farnesene and (*E*)-α-bergamotene, and the homoterpene TMTT (Supplemental Fig. S5).

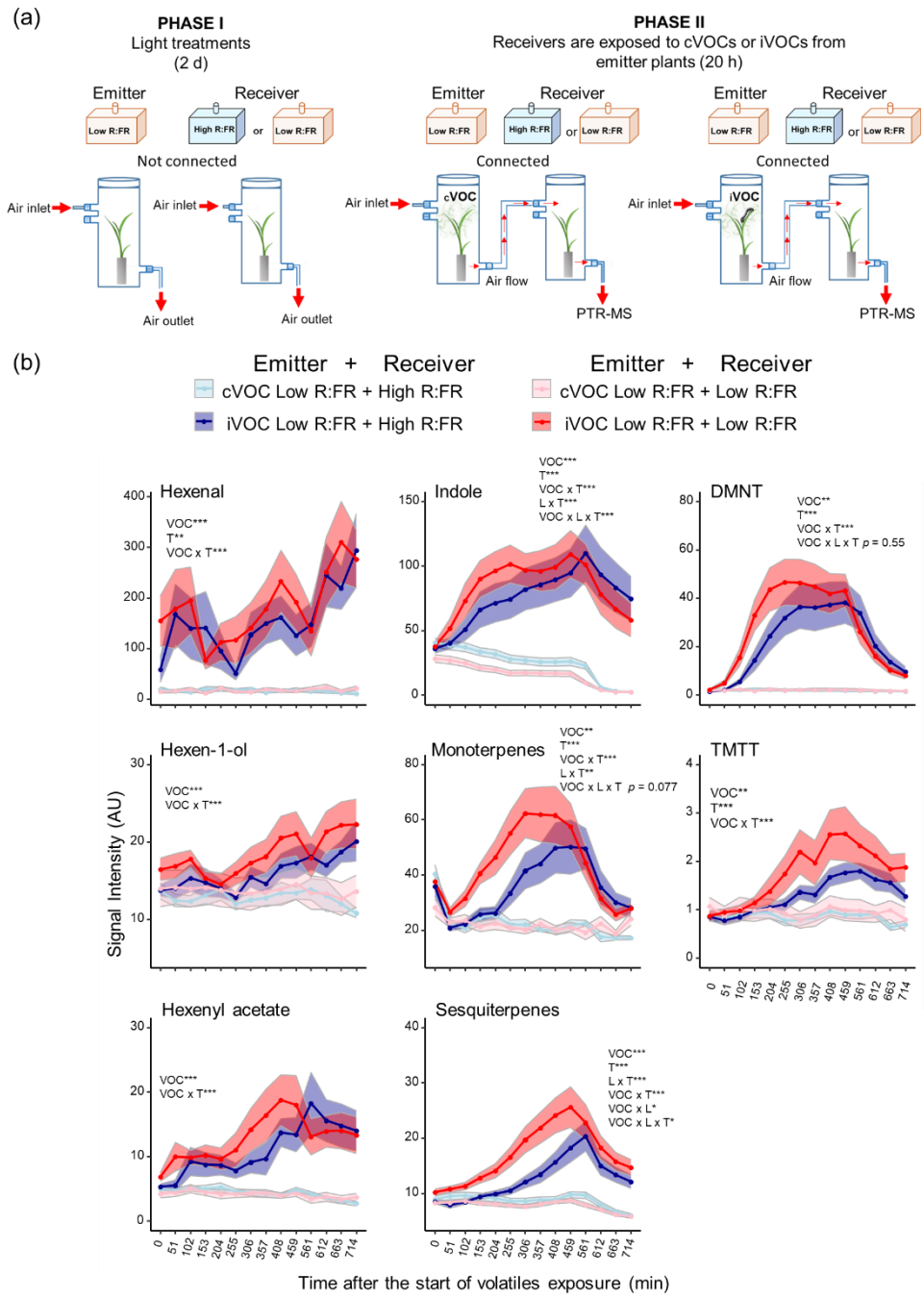

Figure 1. FR-enrichment boosts maize responses to herbivore-induced plant volatiles. (a) Schematic overview of the experimental set-up. Eleven-day old `B73´ maize (*Zea mays*) plants were exposed to low or high R:FR light conditions by modulating FR levels. After two days, receiver plants growing under low or high R:FR were exposed to constitutive volatiles (cVOC) or *S. littoralis* herbivory-induced plant volatiles (iVOC) from emitter plants growing under low R:FR. Emitter and Receiver plants were connected via Teflon tubing and their combined volatile emissions were measured by PTR-TOF-MS. Volatile measurements started at 12:00 a.m. **(b)** Time-series analysis of volatile emissions (mean ± SEM, n = 12) collected from paired plants. The effects of sampling time (T), light treatments of receivers (L), volatiles from emitters (VOC), and their interactions on volatile emissions were tested using linear mixed-effects models. Statistically significant effects are shown in each graph (*$p < 0.05$, ** $p < 0.001$, *** $p < 0.001$). DMNT and TMTT stand for the homoterpenes (*E*)-4,8-dimethyl-1,3,7-nonatriene and (*E*, *E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene, respectively. AU refers to arbitrary units.

Low R:FR alone had a positive effect on the emission of the (*E*)-β-farnesene, and it also increased (*Z*)-3-HAC-induced emission of indole, linalool and (*E*)-α-bergamotene. We did not detect a significant induction of the monoterpenes α-pinene and β-ocimene. Further analyses using dispensers with pure linalool, which is a monoterpene alcohol, showed that the most abundant fragment ion of linalool has an m/z of 137 ($C_{10}H_{17}^{+}$) (Supplemental Fig. S6) like other monoterpenes. Thus, the induced monoterpene signal detected in the PTR-MS experiments upon (*Z*)-3-HAC and FR treatment is likely due to enhanced linalool emissions. FR effects on indole emissions agreed with the enhanced induction observed in Fig. 1b but differed from PTR-TOF-MS data displayed in Fig. 2b, indicating a stronger context-dependent variation in the emission of this compound. Upon simulated herbivory, linalool, (*E*)-β-farnesene, and (*E*)-α-bergamotene emissions were higher under low R:FR than under high R:FR irrespective of (*Z*)-3-HAC pre-treatment. TMTT was below the limit of detection of the GC-MS. During (*Z*)-3-HAC exposure and after simulated herbivory, DMNT emission was higher under low R:FR than under high R:FR, albeit these differences were not statistically significant. This might be explained by the lower number of sampling times compared to the PTR-TOF-MS experiments, resulting in reduced statistical power.

In field-grown maize, heterogeneity in R/FR environments within maize canopies is detected from early ontogenetic stages – i.e., 30 days from sowing - to flowering. This heterogeny is characterized by an enriched FR environment that acts as a signal for leaf reorientation to escape vegetation shade (Maddonni *et al*., 2002). To put our results into a more realistic context, we tested if the observed response to low R:FR and (*Z*)-3-HAC also occurs in 30-day-old B73 maize plants. Low R:FR significantly enhanced monoterpenes, sesquiterpenes, DMNT, and TMTT emissions during (*Z*)-3-HAC exposure, and it did not affect indole (Supplemental Fig. S7). Upon simulated herbivory, pre-exposure to (*Z*)-3-HAC enhanced indole and sesquiterpenes emissions under both light treatments. Irrespective of (*Z*)-3-HAC pre-treatment, low R:FR increased monoterpenes, sesquiterpenes, TMTT, DMNT, and indole emissions upon herbivory. We also tested whether the slight variations in horizontal PAR levels detected in this experimental set up could have influenced maize responses to (*Z*)-3-HAC. There was no significant correlation between PAR and volatile emissions at the sampling time with the strongest (*Z*)-3-HAC effect (Supplemental Fig. S8), confirming FR specific effects on maize responses to (*Z*)-3-HAC.

Together, these results demonstrate that FR-enrichment increases maize responsiveness to (*Z*)-3-HAC, resulting in higher direct and primed emission of terpenes in both 11- and 30-day-old maize plants.

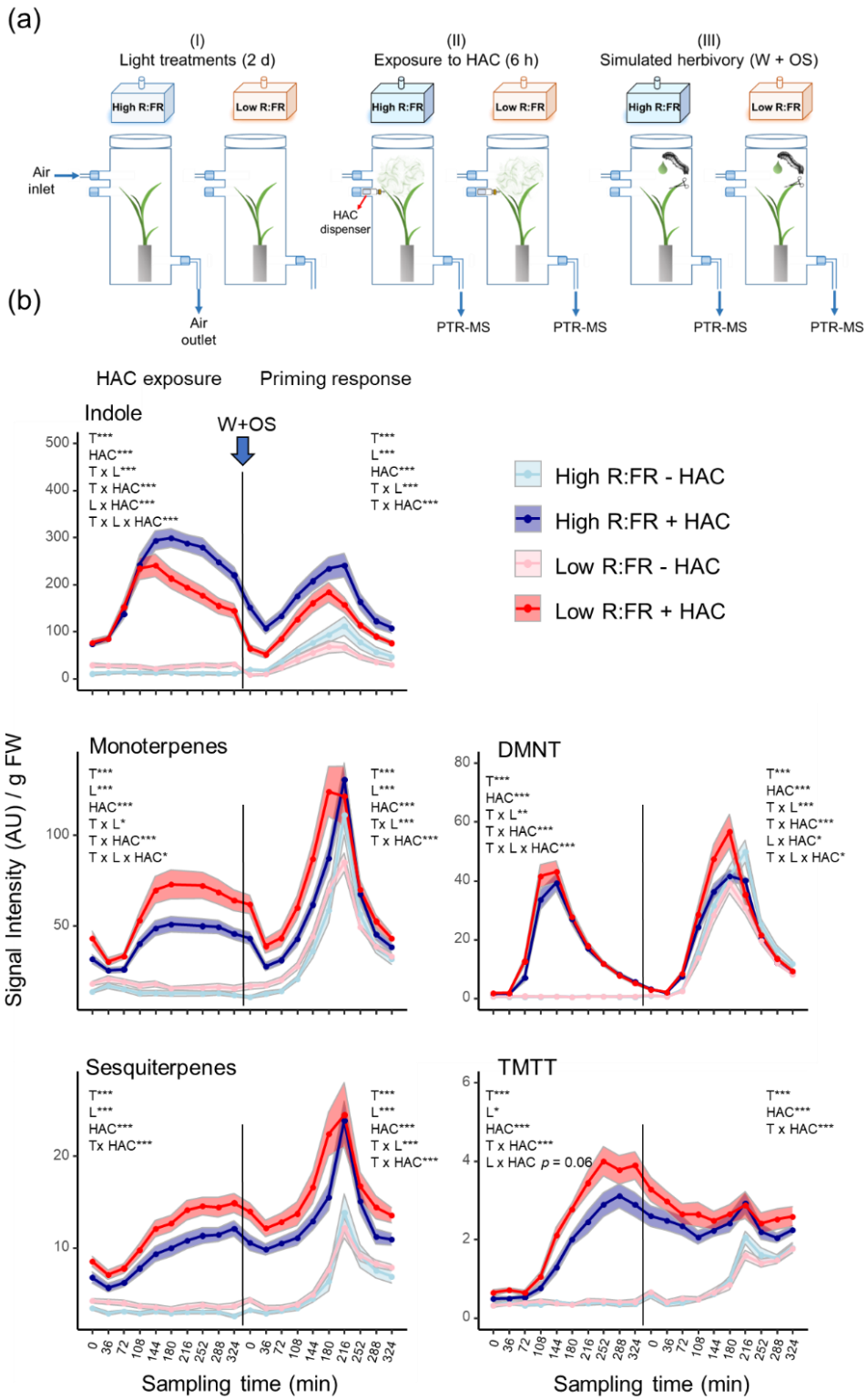

Figure 2. FR-enrichment boosts maize responses to (*Z*)-3-hexenyl acetate. (a) Schematic overview of the experimental set-up. Eleven-day old `B73´ maize (*Zea mays*) plants were exposed to low or high R:FR conditions for 2 d by modulating FR levels, and subsequently exposed to (*Z*)-3-hexenyl acetate (HAC) or left untreated for 6 h. After HAC treatment, plants were induced with simulated herbivory (wounding and application of *Spodoptera littoralis* oral secretions; W+OS). Volatile emissions were determined during HAC exposure and after simulated

herbivory by PTR-TOF-MS. **(b)** Time-series analysis of volatile emissions (mean ± SEM, n = 9) determined in low and high R:FR-treated plants during exposure to HAC (+ HAC) or clean air (- HAC) and after induction with simulated herbivory (priming phase). HAC exposure and volatile measurements started between 9:00 and 10:00 am. The effects of the sampling time (T), HAC, light treatment (L) and their interactions on volatile emissions were tested separately for the HAC exposure and priming phase using linear mixed-effects models. Statistically significant effects are shown in each graph (*p < 0.05, ** p < 0.001, *** p < 0.001). DMNT and TMTT stand for the homoterpenes (*E*)-4,8-dimethyl-1,3,7-nonatriene and (*E*,*E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene, respectively. AU refers to arbitrary units.

Maize volatile emissions in response to (Z)-3-HAC are reduced in the phyB1phyB2 mutant

Low R:FR is sensed by the phytochrome family of photoreceptors, of which phyB is the central regulator in Arabidopsis (Ballaré & Pierik, 2017). In maize, gene duplication has resulted in two PhyB1 and PhyB2 genes, both involved in FR signaling (Sheehan *et al.*, 2007; Dubois *et al.*, 2010). We next determined whether a non-functional phyB enhances maize responses to (*Z*)-3-HAC like low R:FR.

First, we exposed 11-day-old *phyB1phyB2* maize double mutant plants and its wild-type (inbred line W22) to high R:FR for two days and determined volatile emissions during (*Z*)-3-HAC exposure and after simulated herbivory using the PTR-TOF-MS system. Compared to the wild-type, constitutive emission of indole, monoterpenes, sesquiterpenes, and DMNT was significantly lower in *phyB1phyB2* plants (Fig. 3). (*Z*)-3-HAC increased volatile emissions in wild-type and *phyB1phyB2* plants (Fig. 3), but the induction of volatiles was significantly lower in *phyB1phyB2* plants compared to the wild-type. Similar patterns were observed following simulated herbivory (Fig. 3).

To better understand the unexpected response of *phyB1phyB2* plants, we conducted additional experiments. First, we analyzed photomorphogenic responses of *phyB1phyB2* and wild-type W22 plants to low R:FR. As expected, *phyB1phyB2* plants grew longer stem internodes irrespective of the light regime (Supplemental Fig. S9). The wild-type W22 did not show a strong response to low R:FR, which might be explained by maize genotype-dependent responses to supplemental FR (Dubois *et al.*, 2010).

Next, we compared the responsiveness of the wild-type W22 and *phyB1phyB2* plants to (*Z*)-3-HAC when grown under high or low R:FR. Low R:FR significantly enhanced (*Z*)-3-HAC-induced emission of monoterpenes, sesquiterpenes, and DMNT in wild-type W22 plants (Supplemental Fig. S10) as observed before in B73 (Fig. 2b and Supplemental Fig. S5), whereas the effect on indole differed along the time course. Light did not significantly affect volatile emissions upon simulated herbivory. In *phyB1phyB2* plants, low R:FR slightly increased indole, monoterpenes, and sesquiterpenes emissions at the start of the (*Z*)-3-HAC treatment (Supplemental Fig. S11), and it did not significantly affect volatile emissions after simulated herbivory. These experiments indicate that *phyB1phyB2* plants retain partial, but not full, enhanced responsiveness to (*Z*)-3-HAC when simultaneously exposed to low R:FR.

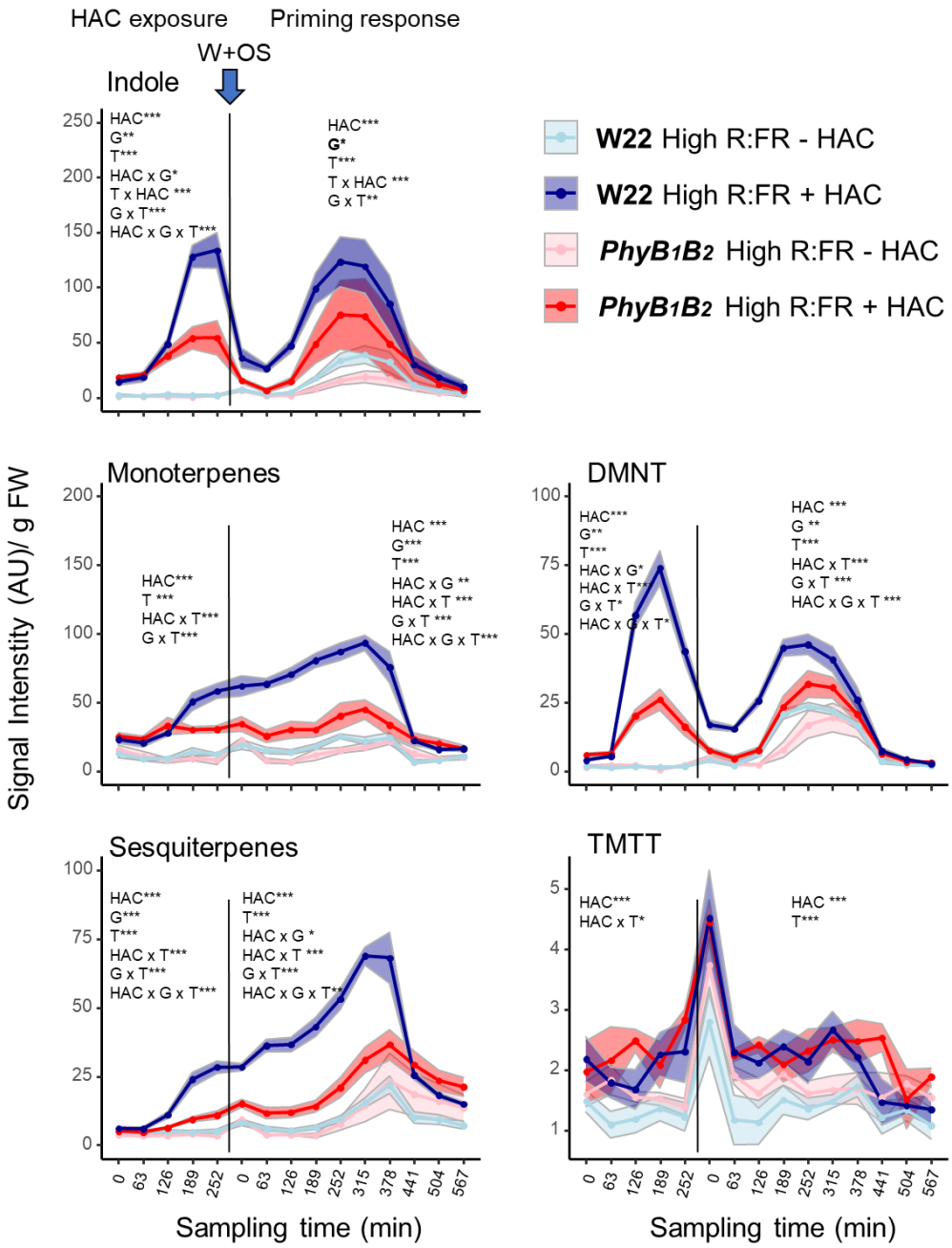

Figure 3. Maize volatile emissions in response to (*Z*)-3-HAC are reduced in the *phyB1phyB2* mutant. Time-series analysis of volatile emissions (mean ± SEM, n = 6) determined by PTR-TOF-MS in high R:FR-treated *PhyB₁B₂* double mutant and wild-type (inbred line `W22´) maize (*Zea mays*) plants during exposure to (*Z*)-3-hexenyl acetate (+ HAC) or clean air (- HAC) and after induction with simulated herbivory (wounding and application of *Spodoptera littoralis* oral secretions; W+OS) (priming phase). HAC exposure and volatile measurements started between 9:00 and 10:00 am. The effects of the sampling time (T), HAC, genotype (G), and their interactions on volatile emissions were tested separately for the HAC exposure and priming phase using linear mixed effects models. Statistically significant effects are shown in each graph (*$p < 0.05$, ** $p < 0.001$, *** $p < 0.001$). DMNT and TMTT stand for the homoterpenes (*E*)-4,8-dimethyl-1,3,7-nonatriene and (*E*, *E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene, respectively. AU refers to arbitrary units.

Maize volatile responsiveness to (Z)-3-HAC is stronger under short-term exposure to FR than under long-term exposure

Why do *phyB1phyB2* mutant plants that behave as if continuously exposed to low R:FR show suppression of (*Z*)-3-HAC-induced volatile emissions? One possible explanation is that phyB is inactivated in *phyB1phyB2* mutant plants over the full course of their development, while in FR experiments plants have a deactivated phytochrome for only two days preceding volatile exposure. To test whether duration of phyB inactivation matters for volatile responsiveness, seven-day-old W22 plants were exposed to low or high R:FR for 6 days, or 4 days of high R:FR followed by 2 days of low R:FR (Fig. 4a). Then, we monitored volatile emissions during (*Z*)-3-HAC exposure and subsequent simulated herbivory using the PTR-TOF-MS system (Fig. 4b).

Wild-type W22 plants exposed to low R:FR for 2 days showed stronger volatile responses to (*Z*)-3-HAC than plants exposed to high R:FR (Fig. 4b) as in previous experiments (Supplemental Fig. S10). Short and long-term exposure to low R:FR induced higher indole, sesquiterpenes, and DMNT emissions, but the inverse pattern was observed for TMTT. Induced sesquiterpenes and DMNT emissions at early times of (*Z*)-3-HAC exposure were lower in plants exposed to low R:FR for 6 days than to shorter low R:FR exposure (2 days) and high R:FR only (Fig. 4b). Upon herbivory, short-term exposure (2 days) to low R:FR also enhanced monoterpenes and sesquiterpenes emissions, but this response was attenuated under long-term low R:FR exposure (6 days). Thus, long-term inactivation of phyB by low R:FR may partially explain the discrepancy between the *phyB1phyB2* double mutant phenotype and short-term FR supplementation experiments. These results also suggest that short- and long-term inactivation of phyB might result in different physiological responses.

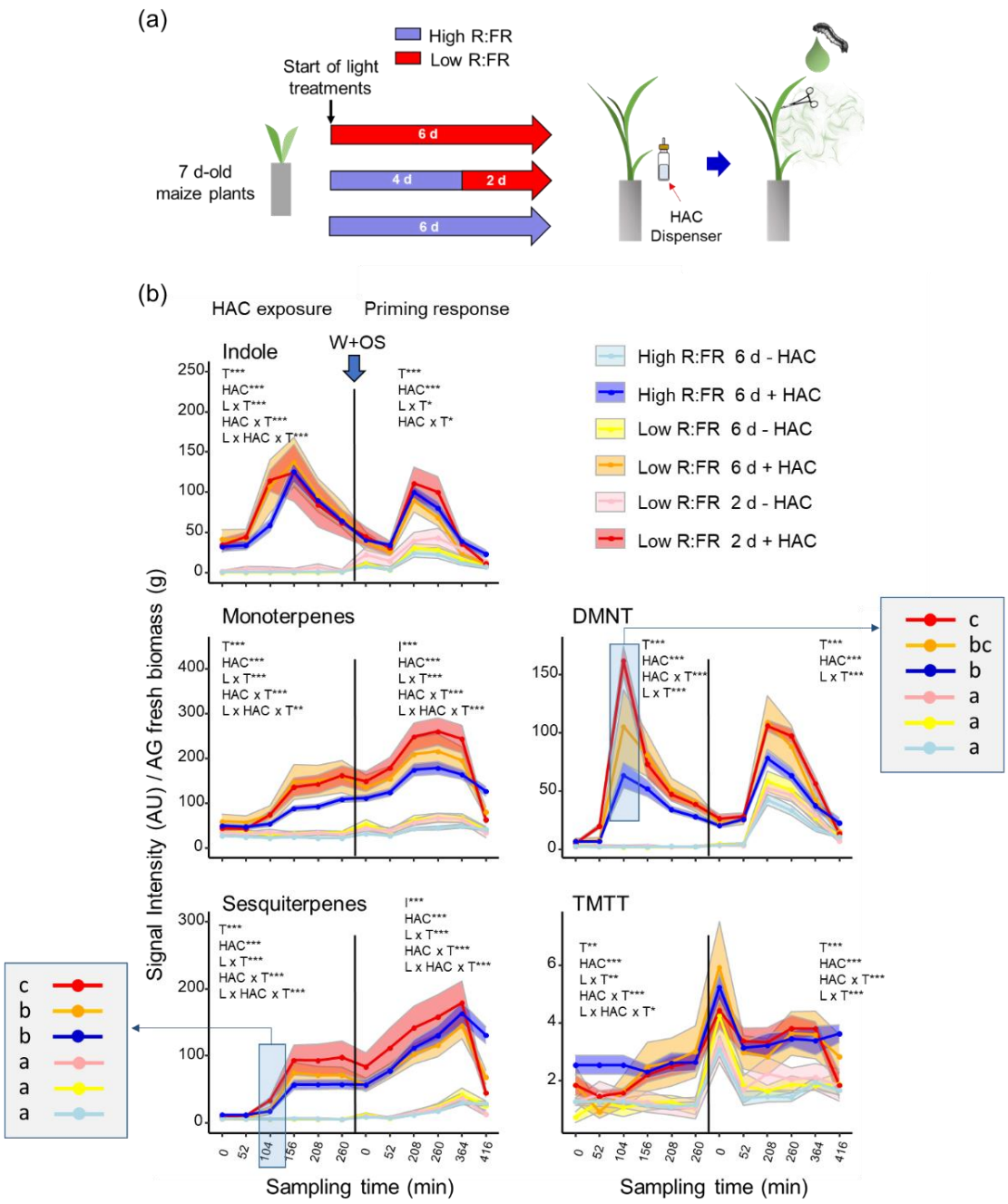

Figure 4. Maize volatile responsiveness to (*Z*)-3-hexenyl acetate is stronger under short- than under long-term FR-enrichment. (a) Schematic overview of the experimental set-up. Seven-day-old W22 maize (*Zea mays*) seedlings were exposed to: (1) high R:FR for 6 days, (2) low R:FR for 6 days, or (3) high R:FR for 4 days and transferred to low R:FR conditions for 2 days. Thereafter, plants were exposed to (*Z*)-3-hexenyl acetate (HAC) for 6 h and subsequently induced with simulated herbivory (wounding and application of *Spodoptera littoralis* oral secretions; W+OS). **(b)** Time-series analysis of volatile emissions (mean ± SEM, $n = 6$) determined by PTR-TOF-MS during exposure to HAC (+ HAC) or clean air (-HAC) and after induction with simulated herbivory (priming phase). HAC exposure and volatile measurements started between 9:00 and 10:00 am. The effects of the sampling time (T), HAC, light treatment (L) and their interactions on volatile emissions were tested separately for the HAC exposure and priming phase using linear mixed effects models. Statistically significant effects are shown in each graph (*$p < 0.05$, ** $p < 0.001$, *** $p < 0.001$). Shaded grey squares containing different letters indicate

significant differences among treatments tested by estimated marginal means at $p < 0.05$. DMNT and TMTT stand for the homoterpenes (*E*)-4,8-dimethyl-1,3,7-nonatriene and (*E*, *E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene, respectively. AU refers to arbitrary units.

FR-enrichment enhances JA-Ile accumulation in response to (Z)-3-HAC

Volatile emissions in maize can be modulated by jasmonates and ABA (Bruinsma *et al.*, 2009; Seidl-Adams *et al.*, 2015). To investigate the potential mechanisms by which short-term exposure to low R:FR enhances volatile emissions, levels of these stress-related hormones were analyzed in 11-day old B73 seedlings exposed to low or high R:FR for two days and subsequently to (*Z*)-3-HAC and simulated herbivory (Fig. 5).

Exposure to (*Z*)-3-HAC increased OPDA, JA, JA-Ile, and ABA levels as previously described (Hu *et al.*, 2019) in both light regimes (Fig. 5a). Light did not affect JA, JA-Ile, and SA levels, but low R:FR slightly reduced OPDA concentrations and enhanced ABA levels. In addition, JA-Ile levels in low R:FR-treated plants at 45 min after the start of (*Z*)-3-HAC exposure were significantly higher when compared to high R:FR-treated plants.

Upon simulated herbivory, (*Z*)-3-HAC pre-treated plants displayed higher OPDA and JA-Ile levels when compared to plants pre-exposed to clean air irrespective of light treatments (Fig. 5b). Low R:FR enhanced JA, JA-Ile, and ABA levels at 180 min after simulated herbivory irrespective of (*Z*)-3-HAC pre-treatment (Fig. 5b).

FR-enrichment modulates the expression of volatile biosynthetic genes in response to (Z)-3-HAC and herbivory

Next, we tested whether low R:FR-induced emission of volatiles in B73 seedlings correlated with changes in the expression of terpene-related biosynthetic genes during (*Z*)-3-HAC exposure and subsequent simulated herbivory in two separate time-series experiments (Fig. 6).

Exposure to (*Z*)-3-HAC significantly induced the expression of *FPSS3,* which provides precursors for sesquiterpene biosynthesis (Richter *et al.*, 2015), *TPS10*, involved in the biosynthesis (*E*)-α-bergamotene and (*E*)-β-farnesene (Schnee *et al.*, 2006), and *TPS2*, involved in the production of linalool, DMNT and TMTT (Richter *et al.*, 2016) (Fig. 6a). *TPS1*, involved in the biosynthesis of (*E*)-β-farnesene, linalool derivatives, and *ent*-kaurene, the latter serving as an intermediate in the production of kauralexins (Schnee *et al.*, 2002; Fu *et al*., 2016; Xu *et al.*, 2019), was also induced by (*Z*)-3-HAC. Expression of *CYP92C5*, involved in the production of DMNT (Richter *et al.*, 2016), was slightly reduced by (*Z*)-3-HAC at 180 min after the start of the treatment (Fig. 6a). (*Z*)-3-HAC effects were not influenced by light. Low R:FR alone, however, reduced the expression of *CYP92C5* and *FPPS3* genes at 45 min and 90 min, respectively, and slightly enhanced *TPS1* expression at 45 min after the start of the volatile treatment (Fig. 6a).

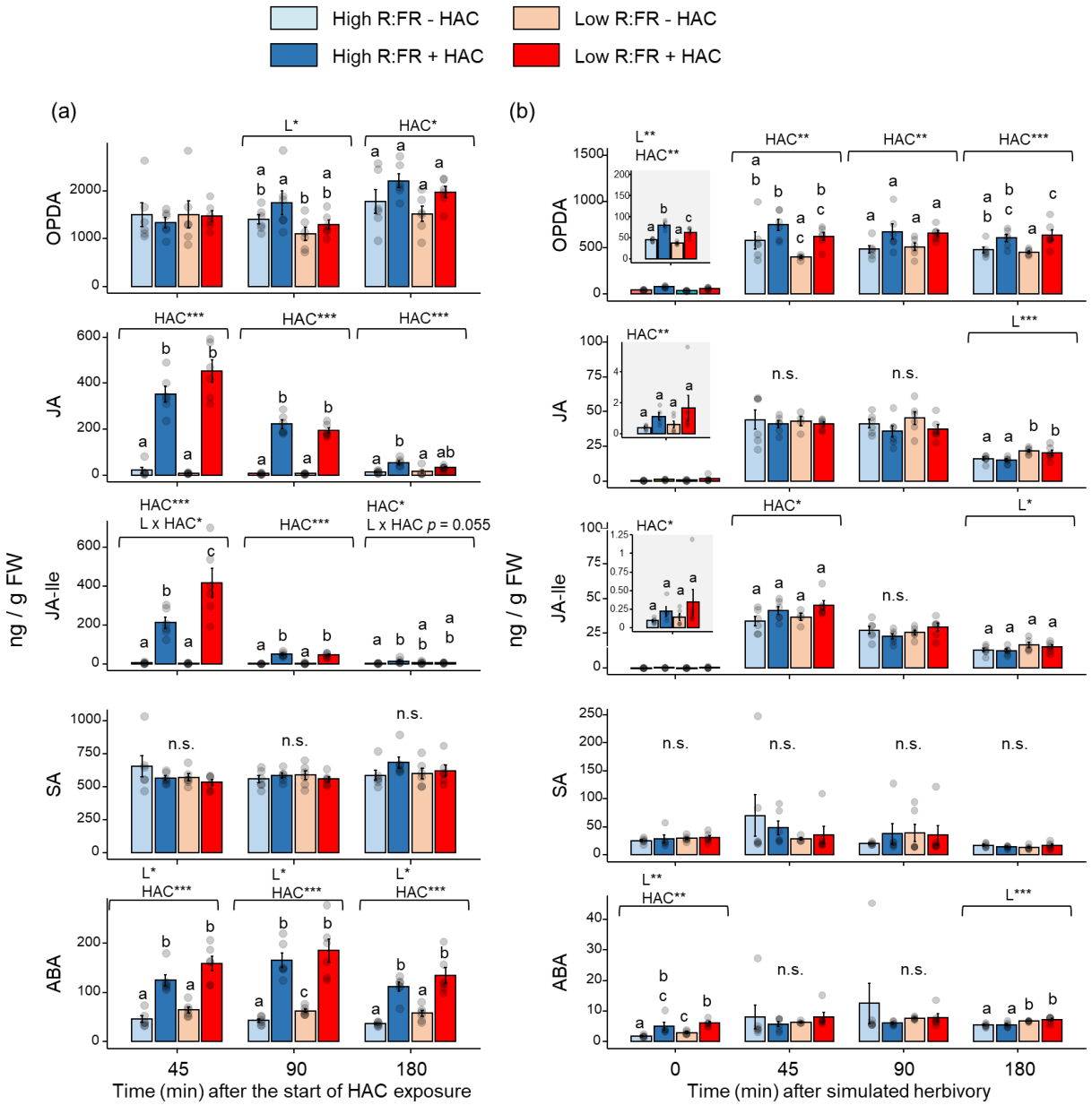

Figure 5. FR-enrichment enhances JA-Ile accumulation in response to (*Z*)-3-hexenyl acetate. Concentrations (mean ± SEM, *n* = 4-6) of 12-oxo-phytodienoic acid (OPDA), jasmonic acid (JA), jasmonic acid-isoleucine (JA-Ile), salicylic acid (SA), and abscisic acid (ABA) in high and low R:FR treated B73 maize (*Zea mays*) plants that were in **(a)** exposed to (*Z*)-3-hexenyl acetate **(**HAC) and in **(b)** induced with simulated herbivory (wounding and application of *Spodoptera littoralis* oral secretions) after exposure to HAC for 6 h. Effects of light treatment (L), HAC exposure and their interaction were tested by two-way ANOVA at each time point. Different letters denote significant differences among groups tested by Tukey-HSD post hoc test. Statistically significant effects are shown in each graph (*$p < 0.05$, ** $p < 0.001$, *** $p < 0.001$).

Upon simulated herbivory, (*Z*)-3-HAC pre-treatment enhanced the expression of *TPS2* and *TPS10* and suppressed *CYP92C5* expression in low and high R:FR-treated plants, with no effect on *FPSS3* and *TPS1* (Fig. 6b). Low R:FR enhanced *TPS2* expression in (*Z*)-3-HAC pre-treated plants and led to a stronger (*Z*)-3-HAC-mediated CYP92C5 suppression upon simulated herbivory. Irrespective of (*Z*)-3-HAC pre-treatment, low R:FR slightly enhanced *TPS2*, *TPS10*, and *CYP92C5* expressions after simulated herbivory (Fig. 6b). These results show that FR-enriched environments and (*Z*)-3-HAC interact to modulate the expression of some, but not all, terpene biosynthesis genes, and that these slight changes are unlikely to fully explain the differences in volatile emissions.

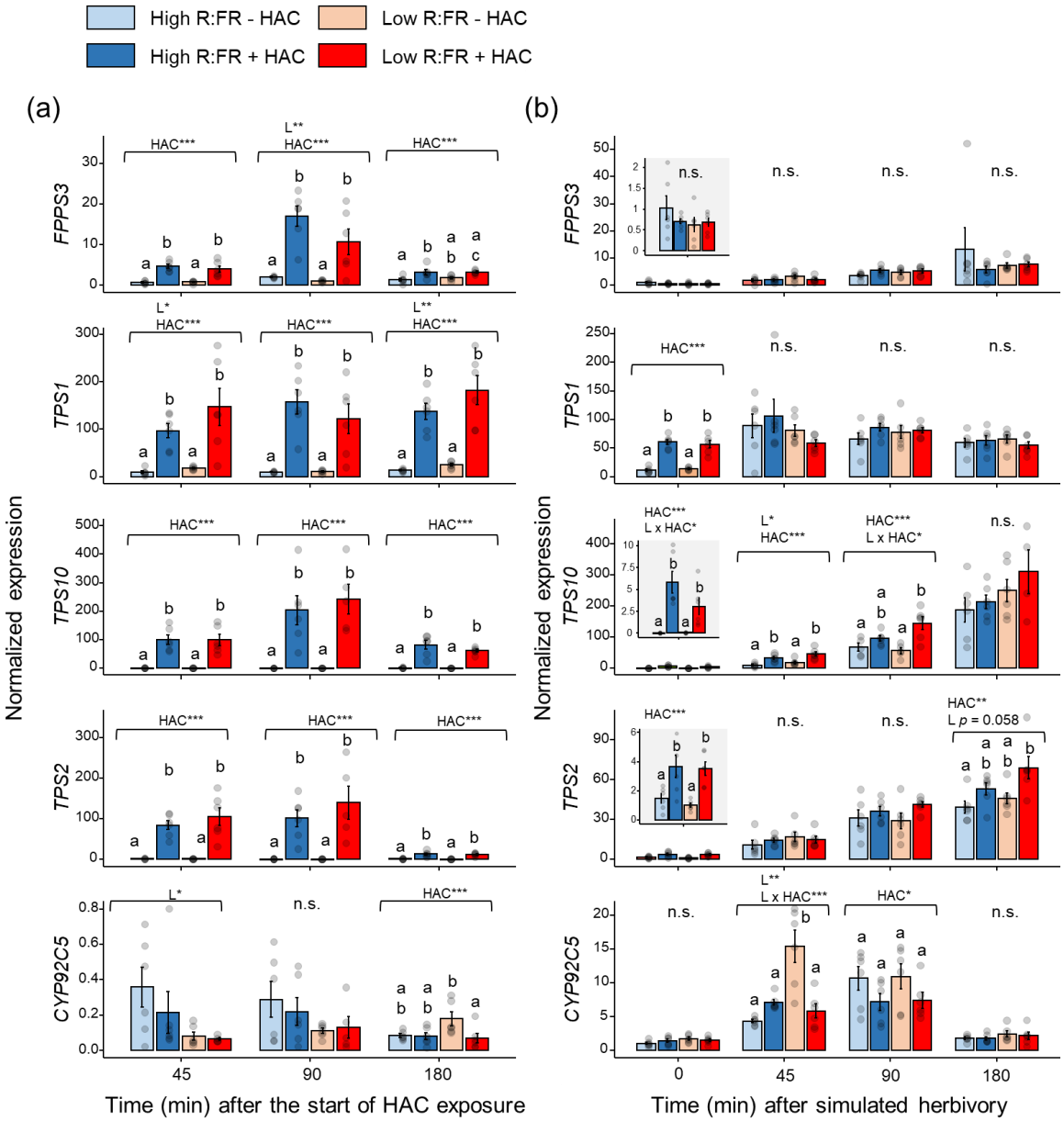

Figure 6. FR-enrichment modulates the expression of volatile biosynthetic genes in response to (*Z*)-3-hexenyl acetate and herbivory. Normalized expression (mean ± SEM, *n* = 6) of terpene metabolism-related

genes determined in aboveground (leaves and stem) tissues of low and high R: FR-irradiated B73 maize (*Zea mays*) plants that were in **(a)** exposed to (*Z*)-3-hexenyl acetate (HAC) and in **(b)** induced with simulated herbivory (wounding and application of *Spodoptera littoralis* oral secretions; W+OS) after exposure to HAC for 6 h. Effects of light treatment (L), HAC and their interaction were tested by two-way ANOVA at each time point. Different letters denote significant differences among groups tested by Tukey-HSD post hoc test. Statistically significant effects are shown in each graph (*$p < 0.05$, ** $p < 0.001$, *** $p < 0.001$) Abbreviations for precursors and genes: *FPPS3*, farnesyl pyrophosphate synthase 3; *TPS1, Terpene synthase 1; TPS10*, *Terpene synthase 10*; *TPS2*, *terpene synthase 2*; *CYP92C5*, *P450 Monooxygenase CYP92C5.*

FR-enrichment increases stomatal conductance and photosynthesis

Emission of sesquiterpenes can be regulated by light-dependent stomata movements in maize (Seidl-Adams *et al.*, 2015). We tested whether differences in stomata conductance accounted for the increase in volatile emissions under low R:FR. For this, 11-day-old B73 seedlings were exposed to low or high R:FR for two days by modulating FR levels, and leaf stomatal conductance was determined at 3 h after (*Z*)-3-HAC exposure and at 4 h after subsequent simulated herbivory.

Exposure to (*Z*)-3-HAC significantly reduced stomatal conductance in maize (Fig. 7ab). Low R:FR significantly increased stomatal conductance (Fig. 7ab), which was not explained by differences in stomatal densities (Supplemental Fig. S12). We did not detect significant interactions between light and (*Z*)-3-HAC on stomatal conductance. Simulated herbivory reduced stomatal conductance close to the wounding sites (Fig. 7cd), a phenomenon driven by OPDA signaling in local leaves of dicotyledonous plants (Savchenko et al., 2014; Meza-Canales et al., 2017). In leaf 2, stomatal closure was slightly stronger in wounded plants exposed to low R:FR and (*Z*)-3-HAC pre-treatment (Fig. 7d).

Stomatal opening as well as volatile biosynthesis are tightly coupled to photosynthetic rates (Arimura *et al.*, 2008; Way & Pearcy, 2012). We therefore evaluated if light and volatile cues interact to regulate photosynthesis. While (*Z*)-3-HAC had no significant effect on photosynthesis, photosynthetic rates were significantly increased under low R:FR in leaf 3 (Fig. 8ab).

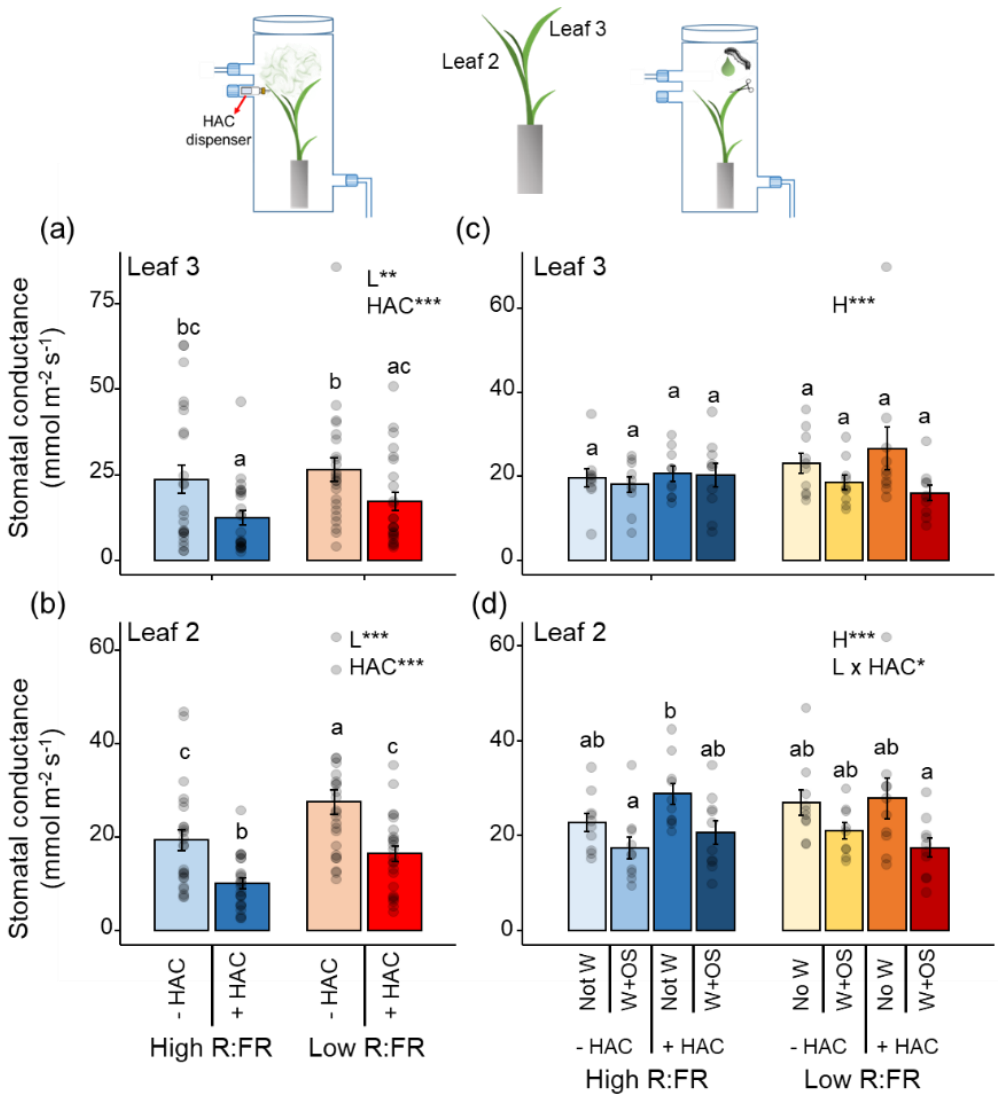

Figure 7. FR-enrichment increases stomatal conductance in maize. Stomatal conductance (mean ± SEM, *n* = 20) determined in the abaxial side of **(a)** leaf #3 and **(b)** leaf #2 from the bottom of low and high R:FR-treated B73 maize (*Zea* mays) plants after 3 h of exposure to (*Z*)-3-hexenyl acetate (HAC). Effects of light treatment (L), HAC exposure and their interaction were tested by two-way ANOVAs. Stomatal conductance (mean ± SEM, *n* = 10) determined in the abaxial side of **(c)** leaf #3 and **(d)** leaf #2 of low and high R:FR-treated B73 plants that were induced with simulated herbivory (wounding and application of *Spodoptera littoralis* oral secretions; W+OS) after exposure to HAC for 6 h. Measurements were performed close to the wounding sites at 4 h after simulated herbivory. Effects of light treatment (L), HAC exposure, herbivory (H), and their interaction were tested by three-way ANOVAs. In all graphs, different letters denote significant differences among groups tested by Tukey-HSD post hoc test. Statistically significant effects are shown in each graph (*$p < 0.05$, ** $p < 0.001$, *** $p < 0.001$).

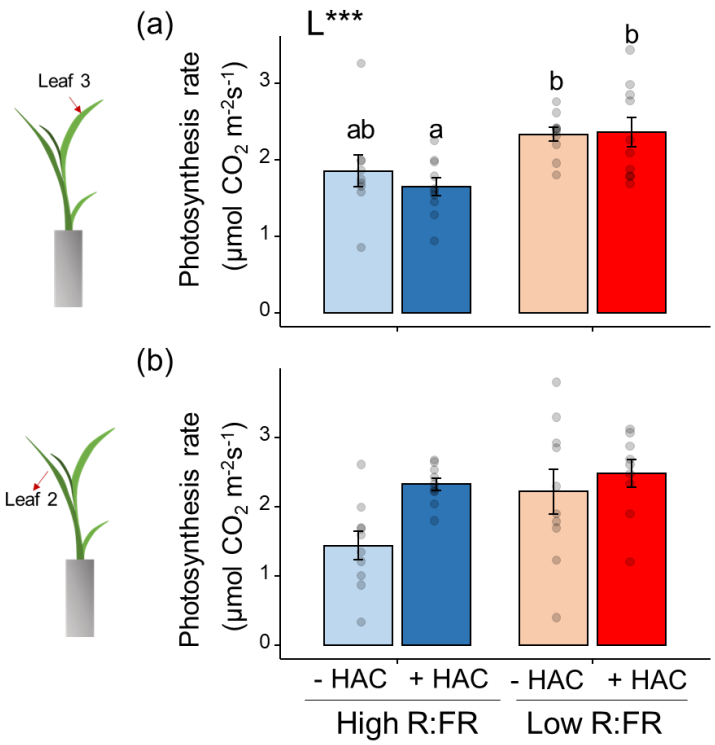

Figure 8. FR-enrichment increases photosynthesis in maize Photosynthesis rate (mean ± SEM, n = 10) determined in the abaxial side of **(a)** leaf 3 and **(b)** leaf 2 from the bottom of high and low R:FR-treated B73 maize plants at 3 h after the start of (*Z*)-3-hexenyl acetate (HAC) exposure. Effects of light treatment (L) and HAC on stomatal density were tested by two-way ANOVAs. Statistically significant effects are shown in the graphs. ***p < 0.001. Different letters denote significant differences among groups tested by Tukey-HSD post hoc test at p < 0.05.

Discussion

In closing or dense canopies, plants can detect the proximity and physiological state of neighboring plants through light and volatile cues. Our results demonstrate that maize plants integrate both stimuli into distinct defense-related volatile signaling patterns. Below, we discuss the potential mechanisms and ecological consequences of this phenomenon.

HIPVs can induce defense-related responses in undamaged plants including the biosynthesis and emission of volatiles (Arimura *et al.*, 2002; Choh *et al.*, 2004; Engelberth *et al.*, 2004) and the priming of defense responses when actual herbivory occurs (Ton *et al.*, 2007; Frost *et al.*, 2008; Erb *et al.*, 2015; Hu *et al.*, 2019; Paudel Timilsena *et al.*, 2020). Here, we report that FR-enriched light environment prompts maize plants to respond more strongly to HIPVs emitted by their neighbors. We found that direct and priming of volatile release in response to HIPVs and (*Z*)-3-HAC is enhanced in plants exposed to FR-enriched conditions, the typical spectral signature of the light environment in closing plant canopies and shaded environments. In line with our findings, Chautá and Kessler (2022) also reported a higher emission of a sesquiterpene in tall goldenrod (*Solidago altissima*) plants

exposed to supplemental FR and HIPVs from neighbors, but the mechanism remained to be determined.

Most of the volatile identities and PTR-TOF-MS emission patterns in the present work were validated using a complementary dynamic volatile collection and GC-MS detection. The higher sensitivity of the PTR-TOF-MS allowed the detection of less abundant volatiles such as TMTT, and it also enhanced the statistical power of the analyses by increasing their temporal resolution. Strikingly, (*Z*)-3-HAC-induced indole emissions showed varying patterns between experiments, being slightly enhanced by FR in some of them and during the time-series analyses while strongly reduced in others (Fig. 2b). Further experimentation is needed to determine the context-depended regulation of this compound emissions.

Plant responses to volatiles are regulated by the jasmonate signaling pathway (Arimura *et al.*, 2000; Hu, 2022) as part of an integrated signaling network that controls plant growth and defense responses (Schmelz *et al.*, 2003; Bosch *et al.*, 2014; Mujiono *et al.*, 2021). In maize, (*Z*)-3-HAC induces the accumulation of jasmonates and expression of JA-related marker genes before and after herbivory induction (Hu *et al.*, 2019). Here, we confirmed that (*Z*)-3-HAC strongly up-regulated JA signaling in maize. Furthermore, we found that low R:FR or FR-enriched environment resulted in a two-fold higher induction of JA-Ile levels. This is surprising considering the reported suppressing effects of low R:FR on JA accumulation (Fernandez-Milmanda et al., 2020) and signaling in Arabidopsis (Cerrudo *et al*., 2012; de Wit *et al*., 2013; Chico *et al.*, 2014; Leone *et al.*, 2014). Yet, low R:FR induced characteristic shade-associated morphological responses in maize, such as the elongation of stems. Furthermore, we observed strong inductions of auxin and ABA levels in low R:FR treated plants, with both hormones involved in shade-induced suppression of branching (tillering in grasses) (Küpers *et al.*, 2020; Fernández-Milmanda & Ballaré, 2021). PAR levels and R:FR ratios used in our work were similar to conditions employed in previous work reporting the effects of supplemental FR on hormonal signaling and defenses in Arabidopsis (Pierik *et al.*, 2009; Wit *et al.*, 2013) and tomato (Courbier *et al.*, 2020; Courbier *et al.*, 2021) Thus, although maize responses to shade show similarities with those described for Arabidopsis and other plant species, there might be significant points of divergence. Additional analyses using reverse genetics and pharmacological approaches can reveal the importance of the enhanced jasmonate response and the potential role of auxin and ABA in maize responses to (*Z*)-3-HAC.

Plant volatile emission is controlled by biochemical, physiological, and physicochemical processes including the rate of volatile biosynthesis, the activities of enzymes, the volatility and diffusion rates of distinct volatile compounds, and stomatal conductance (Niinemets *et al.*, 2004; Seidl-Adams *et al.*, 2015). Our study showed that low R:FR changed the expression of a subset of volatile biosynthesis genes in response to (*Z*)-3-HAC and simulated herbivory. However, the observed interactions were weak and did not track the changes in volatile emissions. Thus, it is unlikely that light

and volatile cues interact at this level to boost volatile release in maize. Low R:FR effects on the diffusion of volatiles through the cuticle is also an unlikely explanation, as the cuticular diffusion coefficients of monoterpenes is lower than the diffusion coefficient of air and water, indicating that the cuticle provides an efficient barrier for these plant volatiles (Niinemets, 2003). We therefore reasoned that volatile emissions might be alternatively modulated by induced changes in stomatal conductance. Our results showed that both exposure to (*Z*)-3-HAC and simulated herbivory reduced leaf stomatal conductance, which contrasted with the strong increase in volatile emissions. This might be explained by the fact that leaf stomata can only limit volatile emission if the decrease in stomatal conductance (i.e., closure) is larger than the increase in the volatile diffusion gradient (Lin *et al.*, 2022). Most probably, (*Z*)-3-HAC induction of volatile biosynthesis quickly increased their concentration in the intercellular air spaces, enhancing volatile diffusion gradients and overruling stomatal limitations.

Interestingly, FR-enriched environment had a positive effect on leaf stomatal conductance. This is consistent with previous findings in bean (*Phaseolus vulgaris*) where supplemental FR added from above to sub-saturating levels of visible light increased stomatal conductance (Holmes *et al.*, 1986). More recently, FR has been shown to enhance photochemical efficiency and photosynthesis in several dicotyledonous and C3 and C4 monocotyledonous species, including maize, when added to a background of white light (Zhen & Bugbee, 2020). In maize, FR photons were recently shown to account for a large fraction of the photosynthetic activity under vegetation shade (Zhen *et al.*, 2022). This was explained by their enhancing linear electron transport efficiency and cyclic electron flow around the photosystem I (Zhen *et al.*, 2022). In C4 species such as maize, this is especially relevant due to the extra ATP requirement for the CO_2-concentrating mechanism in the bundle sheath cells (Johnson, 2011). Hence, our results align with the expected stomatal and photosynthetic responses of maize plants in dense canopies characterized by low irradiances in the visible light range (400-700 nm) and enrichment in the FR region of the spectrum. Zhen and Bugbee (2020) also found that the addition of equal amounts of FR or white photons causes similar increases in net photosynthesis, while the increase in stomatal conductance was comparatively smaller under FR. This suggested that stomatal responses to FR were most likely a response to the increase in photosynthetic rate. Stomatal conductance in Arabidopsis *phyB* mutants have been shown to be modulated by changes in ABA sensitivity under drought stress (González *et al.*, 2012). Whether the increase in stomatal conductance from adding FR result from increased photosynthetic rates and/or of alteration of ABA sensitivity needs to be established. Yet, it is conceivable that the effects of FR-enriched environment on the emission of volatiles in response to (*Z*)-3-HAC might be explained, at least in part, by the positive effects of supplemental FR on stomatal conductance and/or higher photosynthetic rates. The latter might affect the availability of precursors for volatile biosynthesis (Arimura *et al.*, 2008).

Unlike FR supplementation experiments, *phyB1phyB2* plants displayed a diminished response to (*Z*)-3-HAC. These results align with the suppressive effect of phyB inactivation on plant defenses

(Ballaré, 2014; Pierik & Ballaré, 2021). We reasoned that long-term phyB inactivation (as present in the *phyB1phyB2* mutant) may have different effects from those triggered by a short-term inactivation via changes in R:FR. Indeed, longer exposure of maize seedlings to low R:FR resulted in a slightly weaker effect of low R:FR on (*Z*)-3-HAC-induced volatile emissions. At the same time, the *phyB1phyB2* mutant retained some ability to respond to low R:FR with higher (*Z*)-3-HAC induced volatile emissions, reinforcing the potential effects of FR on the photosynthetic machinery and stomatal conductance. We therefore conclude that (i) the duration of phyB inactivation might explain FR effects on maize responses to (*Z*)-3-HAC, and that (ii) an enhanced photosynthetic efficiency and/or (iii) other FR photoreceptors may also play a role in this phenomenon. Under deep shade (R:FR < 0.8), phyA is strongly activated and prevents the excessive elongation of seedlings in Arabidopsis, antagonizing phyB FR-induced responses (Casal *et al.*, 2014; Martínez-García *et al.*, 2014; Lim *et al.*, 2018; Yang *et al.*, 2018). Furthermore, PhyA promotes photosynthetic processes in rice (Panda *et al*., 2023). PhyA therefore represents a candidate that may be involved in the responses to light and volatile cues in maize.

From a biological perspective, the capacity to integrate light and volatile cues would seem particularly beneficial to fend off an attacking herbivore. While induced volatiles can serve as reliable cues for the presence of herbivores on neighboring plants (Moreira *et al.*, 2018; Meents & Mithöfer, 2020), light cues can provide information about the proximity of neighbors and, therefore, the risk of being attacked next. Because the effects of herbivore attack are more severe under competition (Vries *et al.*, 2018), it might be advantageous for a plant to respond more strongly to induced volatiles in the presence of neighboring plants and vegetative shade. The enhanced emission of volatiles might, for instance, promote the attraction of natural enemies in a setting where their recruitment is a competitive struggle (Kessler & Baldwin, 2001; Cortés et al., 2016; Aartsma *et al.*, 2017). Plant volatiles may also decrease ovipositing of gravid female moths (Moraes *et al.*, 2001). However, these volatiles may also attract herbivores themselves (Mérey *et al.*, 2013), in which case becoming “apparent” in a dense stand would be the wrong strategy. Further studies on the adaptive benefits of volatile responses in dense stands will help us to understand the evolutionary and adaptive character of plant responses to light and volatile cues in agricultural and ecological settings. Clearly, the integration of light and volatile cues deserves attention as a potentially major determinant of plant-plant, plant-herbivore, tri-trophic interaction dynamics, and community structure.

Acknowledgments

We thank Prof. Dr. Tobias Züst for his help in the optimization of GC-MS protocols and the identification of maize volatile compounds. We also thank Prof. Dr. Christian Fankhauser and Dr. Martina Legris for their help with light spectral measurements. The work of BCJS was supported by the Marie Sklodowska-Curie Action Individual Fellowship (European Union Horizon 2020, Grant Nr. 794,947).

The work of LW was supported by the Marie Sklodowska-Curie Action Individual Fellowship (European Union Horizon 2020, Grant Nr. 886651). This work was supported by the Velux Foundation (Grant Nr. 1231), the Swiss National Science Foundation (Grant. Nr. 200355), the State Secretariat for Education, Research, and Innovation SERI (Project CANWAS) and the University of Bern.

Author contributions

ME conceived the study. ME, RE-B, BCJS, CB, and CAMR designed experiments. RE-B conducted the experiments. RE-B performed the PTR-TOF-MS measurements and analyses. LW assisted with PTR-TOF-MS measurements and conducted the analysis of linalool emissions. RE-B and BCJS conducted gene expression analyses. RE-B and GG performed hormone analyses. RE-B and YZ conducted stomatal measurements. RE-B analyzed the data. RE-B, BCJS, CB and ME interpreted the data. RE-B and ME wrote the manuscript and all the authors contributed to revisions.

Data availability

The data generated for this manuscript will be archived in Dryad and the data DOI will be included at the end of the article.

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Supporting Information

Figure S1. Light spectral composition determined in FR supplementation experiments.

Figure S2. Effects of FR-enrichment on maize growth and auxin levels.

Figure S3. Aboveground biomass in maize plants infested with *Spodoptera littoralis* larvae.

Figure S4. Effect of FR-enrichment on herbivory-induced plant volatile emission in maize

Figure S5. Effect of FR-enrichment on maize volatile emission during exposure to (*Z*)-3-hexenyl acetate and after simulated herbivory measured via GC-MS methods.

Figure S6. Measurement of pure linalool in the PTR-TOF-MS system.

Figure S7. Effect of FR-enrichment and (*Z*)-3-hexenyl acetate on volatile emissions in 30-day-old maize plants.

Figure S8 Spatial variations in photosynthetically active radiation (PAR) levels in the experimental set up did not influence 30-day-old maize responses to (*Z*)-3-hexenyl acetate.

Figure S9. Effect of FR-enrichment on the growth of *phyB1phyB2* maize double mutant and its wild-type.

Figure S10. Effect of FR-enrichment on volatiles emission during exposure to (*Z*)-3-hexenyl acetate and after simulated herbivory in the maize inbred line W22.

Figure S11. Effect of FR-enrichment on volatiles emission during exposure to (*Z*)-3-hexenyl acetate and after simulated herbivory in the *phyB1phyB2* maize double mutant.

Figure S12. Effect of FR-enrichment and (*Z*)-3-hexenyl acetate on stomatal density.

Supplementary Method S1. Detailed protocol for phytohormone extraction and analysis.

Supplementary Table S1. Primer's specifications for gene expression analyses