Maize integrates light and volatile cues from neighboring plants into unique defense responses

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Abstract

- Plants perceive the presence and defense status of their neighbors through light and volatile cues. How plants integrate these stimuli into adaptive responses is not well understood.
- We investigated whether perception of vegetation through the reduction in Red to Far Red light (R:FR) ratios affects maize (*Zea mays*) responses to the herbivore-induced plant volatile (*Z*)-3-hexenyl acetate. By supplementing FR light and using a *phyB1phyB2* maize mutant, we modulated plant light signaling and perception and determined defense responses, including stress hormone deployment and volatile release.
- Exposure to (*Z*)-3-hexenyl acetate induced maize volatile release. FR supplementation increased this response. FR also boosted (*Z*)-3-hexenyl acetate induced JA-Ile levels and promoted stomatal opening and photosynthesis. FR effects were most pronounced after 2 days of light treatment, becoming weaker with longer exposures. In contrast to FR supplementation, the *phyB1phyB2* mutant displayed reduced volatile responsiveness. Comparative analyses with wheat and tomato revealed that the integration of light and volatile cues into enhanced volatile-release is species-specific.
- In conclusion, FR light enhances maize volatile emissions in response to stress volatiles, likely via stronger hormonal signaling and enhanced photosynthesis. Species-specific integration of light and volatile cues into distinct defense responses may have important consequences for plant-plant and plant-herbivore dynamics.

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 different environmental cues to detect biotic stressors, much less is known about their capacity to integrate multiple types of cues into distinc responses (Nguyen *et al.*, 2016; Escobar-Bravo *et al.*, 2017).

Plants have the capacity to 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. A reduction in R to FR ratios (R:FR) is a cue for the presence of other plants' proximity and foliar shade (Ballaré *et al.*, 1990). Plants can respond to reduced R:FR ratios 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 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 responding to changes in the light spectrum, plants can also 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 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, for instance, 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 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-hexenyl acetate can directly induce defenses, volatile biosynthesis, and release (Engelberth *et al.*, 2004; Hu *et al.*, 2019).

Recent work has demonstrated that R:FR ratios can influence defense deployment in plants, including volatiles. Reduced R:FR has been shown to suppress 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. Constitutive volatiles emitted by barley (*Hordeum vulgare*), for instance, show pronounced quantitative changes upon FR supplementation, with reduced emission of several terpenes and increased emission of the monoterpene linalool oxide (Kegge *et al.*, 2015). Similarly, reduced R:FR in combination with shading (i.e., low PAR) reduces the emission of constitutive and methyl jasmonate (MeJA) induced terpenes and green leaf volatiles 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? 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, which generally requires a combination of environmental cues such as temperature, moisture and light to be initiated (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 cues such as light and volatiles into unique and potentially adaptive responses. For instance, plants might reduce their response to volatile cues when they perceive light cues that indicate the presence of neighboring plants as it may reduce the risk of an individual plant being attacked (Barbosa *et al.*, 2009). HIPVs in combination with the disappearance of neighbor-associated light cues on the other hand may indicate that a neighbor is being consumed entirely, thus warranting defense activation.

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 plants integrate light and volatile cues into defense responses. We modulated R:FR ratios as a major light cue for vegetative shade and (*Z*)-3-hexenyl acetate as a major defense-inducing volatile, and determined volatile emissions, defense gene expression, and phytohormone accumulation in response to combinations of these cues in maize (*Zea mays*). To gain insights into the mechanisms underlying the observed plant responses, we used maize $phyB_1phyB_2$ double mutants, which are impaired in the perception of R:FR light cues. Collectively, our experiments demonstrate that maize plants integrate light and volatile cues into distinct defense responses, thus 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 wheat (*Triticum aestivum*) cultivar 'Fiorina' and the cultivated tomato (*Solanum lycopersicum*) commercial variety 'Moneymaker'. The maize *phyB$_1$phyB$_2$* double mutant was kindly 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*. The allele *phyb1-563* contains a Mu insertion in exon 1 within the phytochromobilin chromophore attachment site and results in no detectable PHYB protein (Sheehan *et al.*, 2007), and the allele *phyb2-12058* contains a Mu transposon in exon 1 that is located 5′ of the chromophore attachment site (Dubois *et al.*, 2010). Seeds were sown in transparent cylindric plastic pots (11 cm height x 4 cm diameter) filled with commercial soil (Selmaterra, BiglerSamen, Switzerland) and wrapped with aluminum foil to prevent root exposure to light. Plants were grown in a ventilated greenhouse supplemented with artificial lighting under a 14/10 h light/dark photoperiod.

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, each containing 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) Red to Far Red ratios (R:FR) (R, λ = 600-700 nm; FR, λ = 700-800 nm) through 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 start of the volatile exposure treatments.

(Z)-3-Hexenyl acetate exposure treatment

Plants were exposed to (*Z*)-3-hexenyl acetate for 6 h within the transparent cylindrical glass chambers of the custom-made high-throughput phenotyping platform described above. One (*Z*)-3-hexenyl acetate dispenser per plant was placed inside the glass chamber. (*Z*)-3-hexenyl acetate dispensers were prepared as described in Hu *et al.*, 2019, each releasing approximately 70 ng h^{-1}. This corresponds to emission levels previously described in herbivore-attacked maize plants (Mérey *et al.*, 2011). (*Z*)-3-hexenyl acetate treatments started between 9:00-10:00 a.m.

Simulated herbivory

Simulated herbivory consisted of wounding two leaves (leaf 2 and 3 from the bottom) 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 has been reported to induce plant defense responses comparable to real herbivory (Erb *et al.*, 2009).

PTR-TOF-MS volatile emission 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 (Vocus PTR-TOF-MS) system (Tofwerk, Switzerland) for analysis. The Vocus PTR-TOF was operated using H_3O^+ as the reagent ion and is 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 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-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). Extracts were then 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) as well as 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 (the 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 measurements were taken 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) and 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 treatment, (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.

Individual plants were included in the model as a random factor and a correlation structure when autocorrelation among residuals was found to be significant ($p < 0.05$). 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. Time-series analysis of hormone concentrations and gene expression were analyzed separately for each time point by two-way ANOVAs followed by Tukey HSD post hoc test when any of the factors, i.e., light treatment, (*Z*)-3-hexenyl aetate and their interaction, were found significant. Effects of light treatments, (Z)-3-hexenyl acetate exposure, herbivory and their interactions on stomatal conductance, density and net photosynthesis in the different experiments were tested by two-way ANOVAs followed by Tukey HSD post hoc test.

Results

Supplemental FR boosts maize responses to herbivore-induced plant volatiles

HIPVs can trigger 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 a FR-enriched light environment alters growth and defense responses to HIPVs in maize, we exposed eleven-day old B73 seedlings to high or low R:FR for two days. Thereafter, we measured growth-related parameters and subsequently compared their volatile emissions when exposed to volatiles emitted by non-infested or *Spodoptera littoralis*-infested low R:FR treated maize plants. For this, emitter plants were infested with ten second-instar *S. littoralis* larvae or left uninfested, and immediately connected to the glass chambers of receiver plants using Teflon tubing (Fig. 1a). 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 Vocus PTR-TOF-MS system.

Low R:FR exposure for two days resulted in elongated stem internodes and enhanced 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, linalool, 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, sesquiterpenes and linalool emissions were significantly higher when receiver plants were exposed to low R:FR and volatiles from *S. littoralis*-infested emitter plants (Fig. 1b). A similar pattern was also 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 cues from the receiver plants, these differences must

be the result of changes in the receivers' volatile emissions. Thus, low R:FR enhances maize responses to HIPVs emitted by herbivore-infested neighbors.

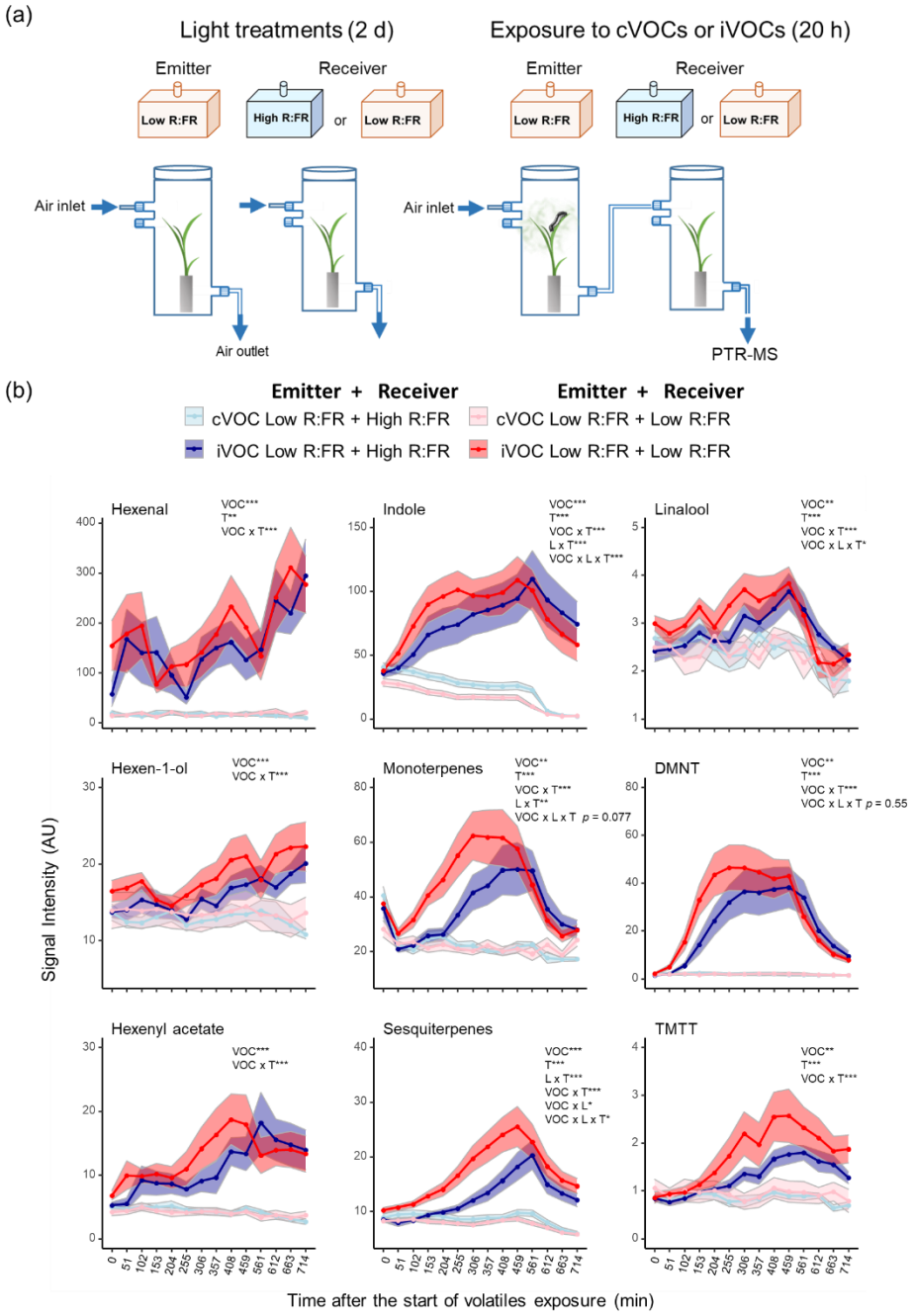


Figure 1. Supplemental FR boosts maize responses to herbivore-induced plant volatiles. (a) Schematic overview of the experimental set-up. Eleven-day old `B73´ maize (*Zea mays*) plants were exposed to low or high R:FR light conditions. After two days, receiver plants growing under low or high R:FR were 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-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 stands 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.

Supplemental FR boosts maize responses to (Z)-3-hexenyl acetate

GLVs such as (*Z*)-3-hexenal, (*Z*)-3-hexen-1-ol, and (*Z*)-3-hexenyl acetate are components of the herbivore-induced volatile blend emitted by maize and known to induce the emission of volatiles in nerighboring intact maize plants (Farag & Paré, 2002; Engelberth *et al.*, 2004). We next tested whether a FR-enriched light environment modulates maize responses to GLVs. We exposed eleven-day old B73 maize seedlings to low or high R:FR for two days and determined their volatile emissions by using the Vocus PTR-TOF-MS system during exposure to (*Z*)-3-hexenyl acetate and subsequent simulated herbivory (Fig. 2a). These analyses were focused on indole and terpenoid emissions, as these compounds were found to respond to low R:FR and herbivory-induced plant volatiles in the previous experiment.

(*Z*)-3-Hexeny acetate directly induced the emission of volatiles in maize seedlings under both low and high R:FR light conditions (Fig. 2b). (*Z*)-3-Hexeny acetate-induced emissions of monoterpenes and DMNT were significantly higher in plants exposed to low R:FR, while the opposite was observed for indole.

Upon wounding and application of *S. littoralis* oral secretions, plants previously exposed to (*Z*)-3-hexenyl acetate exhibited enhanced volatile emissions (Fig. 2b). Induced emission of DMNT after herbivory was significantly higher in low R:FR-treated plants pre-exposed to *Z*)-3-hexenyl acetate than in plants grown under high R:FR. Irrespective of (*Z*)-3-hexenyl acetate pre-treatment, herbivory-induced plants grown under low R:FR showed higher emissions of indole, monoterpenes, sesquiterpenes and DMNT compared to plants grown under high R:FR (Fig. 2b). An additional experiment confirmed that low R:FR enhances the herbivore-induced emission of monoterpenes, linalool, and DMNT, and tends to suppress the emission of indole (Supplemental Fig. S4).

The Vocus 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. Low R:FR significantly enhanced the emission of indole, the monoterpene linalool and the sesquiterpene (E)-α-bergamotene in response to (*Z*)-3-hexenyl acetate (Supplemental Fig. S5). In addition, the emission of the sesquiterpene (E)-β-farnesene was higher under low R:FR. Upon simulated herbivory, linalool, (E)-β-farnesene and (E)-α-bergamotene emissions were higher under low R:FR irrespective of the (*Z*)-3-hexenyl acetate pre-treatment. TMTT was below the limit of detection for the GC-MS.

Together, these results demonstrate that low R:FR, or FR-enriched light conditions, increases maize responsiveness to (*Z*)-3-hexenyl acetate, resulting in higher direct- and herbivore-induced emission of terpenes.

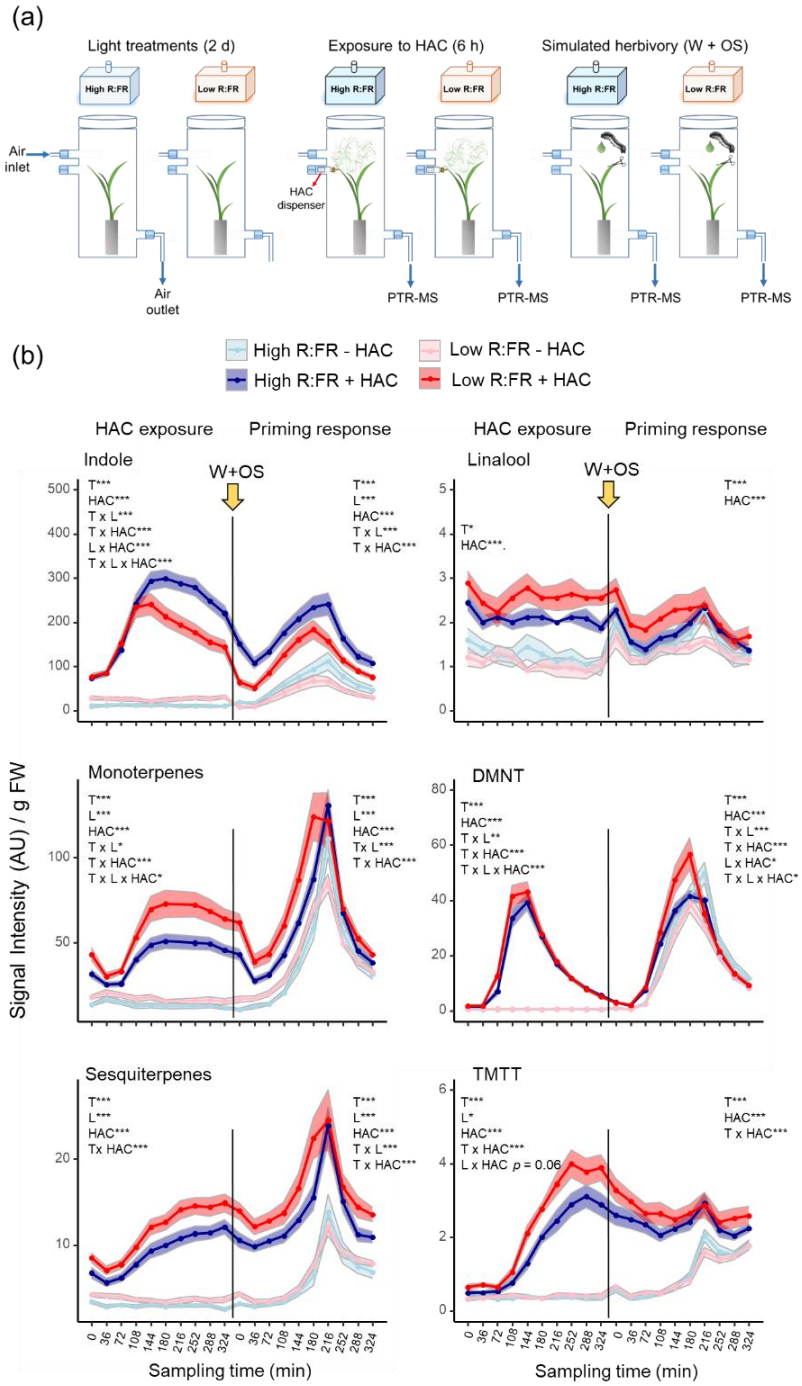


Figure 2. Supplemental FR boosts maize responses to (Z)-3-hexenyl acetate. (a) Schematic overview of the experimental set-up. Eleven-day old `B73´ maize (*Zea mays*) plants were exposed to low or high R:FR conditions for 2 d, and subsequently exposed to (Z)-3-hexenyl acetate (HAC) or left untreated for 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-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 stands 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.

Inactivation of phyB reduces rather than enhances maize volatile emissions in response to (Z)-3-hexenyl acetate

Low R:FR can be sensed by the phytochrome family of photoreceptors, of which phyB is the central regulator in Arabidopsis (Ballaré, 2009). 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-hexenyl acetate like low R:FR ratios.

In a first experiment, we exposed eleven-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-hexenyl acetate exposure and after simulated herbivory using the Vocus 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). The opposite was observed for linalool. (*Z*)-3-hexenyl acetate exposure increased volatile emissions in both wild type and *phyB1phyB2* plants (Fig. 3). However, contrary to our expectation, the induction of volatiles was significantly lower in *phyB1phyB2* plants compared to the wild type W22. A similar pattern was observed following simulated herbivory (Fig. 3).

To better understand the unexpected response of *phyB1phyB2*, we conducted a series of additional experiments with different R:FR ratios. First, we analyzed photomorphogenic responses of *phyB1phyB2* plants and its wild type W22 to low R:FR. As expected, *phyB1phyB2* plants constitutively grew longer stem internodes irrespective of the light regime (Supplemental Fig. S6). The wild type W22 did not show a strong response to low R:FR, which might be explained by previously reported maize genotype-dependent responses to supplemental FR (Dubois *et al.*, 2010). Next, we compared to responsiveness of wild type and *phyB1phyB2* plants to (*Z*)-3-hexenyl acetate when grown under high or low R:FR ratios. Low R:FR significantly enhanced (*Z*)-3-hexenyl acetate-induced emission of monoterpenes, sesquiterpenes, linalool and DMNT in wild type W22 plants (Supplemental Fig. S7) as observed before in B73 (Fig. 2b). In *phyB1phyB2* plants, low R:FR also increased the emissions of indole, monoterpenes and sesquiterpenes at the start of the (*Z*)-3-hexenyl acetate treatment (Supplemental Fig. S8). This effect decreased over time and was not statistically significant at later

timepoints, nor after simulated herbivory. Low R:FR did not significantly affect indole, monoterpenes, sesquiterpenes, and DMNT emissions after simulated herbivory in *phyB1phyB2* plants pre-treated with (*Z*)-3-hexenyl acetate. These experiments indicate that *phyB1phyB2* mutant plants retain partial, but not full, responsiveness to low R:FR ratios when perceiving (*Z*)-3-hexenyl acetate.

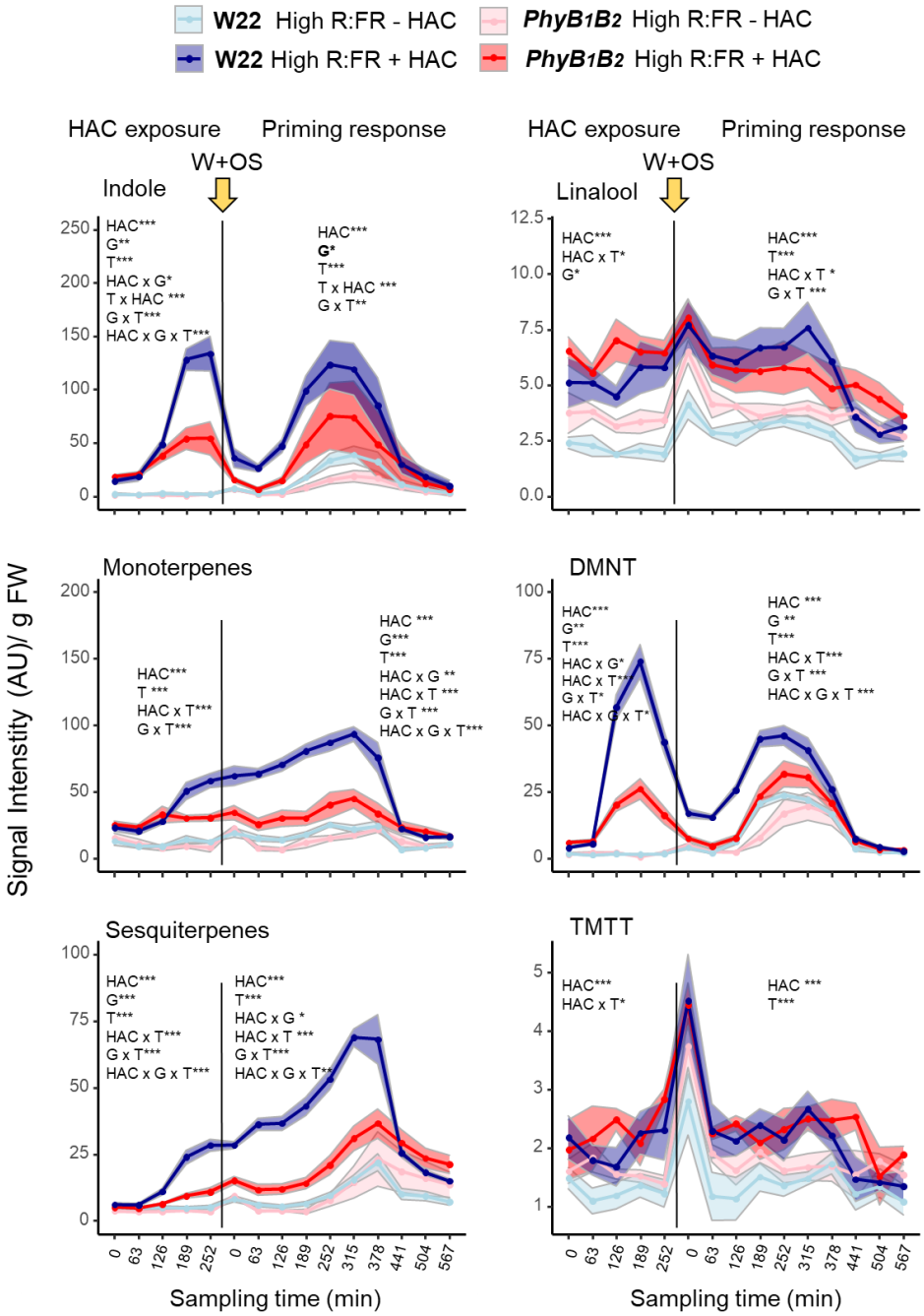


Figure 3. Inactivation of phyB reduces rather than enhances maize volatile emissions in response to (Z)-3-hexenyl acetate. Time-series analysis of volatile emissions (mean ± SEM, *n* = 6) determined by PTR-MS in high R:FR-treated *PhyB₁B₂* double mutant and wild-type (inbred line `W22´) maize (*Zea mays*) 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), 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 stands 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.

Enhancement of maize volatile responsiveness is specific to short-term exposure to supplemental FR

Why do *phyB1phyB2* mutant plants that are at least partially insensitive to changes in R:FR ratios, and behave as if continuously exposed to low R:FR, show suppression of (*Z*)-3-hexenyl acetate-induced volatile emissions? One possible explanation is that *phyB1phyB2* mutant plants have an inactivated phytochrome over the full course of their development, while in our experiments maize plants only have a deactivate phytochrome for two days preceding volatile exposure. To test whether duration of phytochrome deactivation matters for volatile responsiveness, we exposed wild type W22 plants to high R:FR or to low R:FR for 2 or 6 days (Fig. 4a). We then monitored their volatile emissions during (*Z*)-3-hexenyl acetate exposure and subsequent simulated herbivory using the Vocus PTR-TOF-MS system (Fig. 4b).

Wild type W22 plants exposed to low R:FR for 2 days showed a stronger volatile response to (*Z*)-3-hexenyl acetate than plants growing under high R:FR (Fig. 4b), similar to what was observed for B73 (Fig. 2) and in earlier experiments (Supplemental Fig. S7). (*Z*)-3-hexenyl acetate induced the emissions of indole, monoterpenes and sesquiterpenes, except for TMTT, which showed an inverse pattern at earlier time points. Upon herbivory, short-term exposure (2 days) to low R:FR also enhanced the emission of monoterpenes, sesquiterpenes, and linalool. These patterns were attenuated in W22 plants growing for 6 days under low R:FR ratios. Emissions of sesquiterpenes and DMNT were statistically similar between high R:FR-treated plants and plants exposed to low R:FR for 6 days at early times after the start of (*Z*)-3-hexenyl acetate (Fig. 4b). This suggests that long-term inactivation of phyB by reduced R:FR attenuates (*Z*)-3-hexenyl acetate-induced volatile emission, and that enhanced maize responses to HIPVs under low R:FR are phyB-independent. Thus, low R:FR ratios enhance responsiveness to (*Z*)-3-hexenyl acetate in the short, but less in the long run, which may explain why *phyB1phyB2* mutant plants do not show enhanced responsiveness to (*Z*)-3-hexenyl acetate when growing under high R:FR conditions.

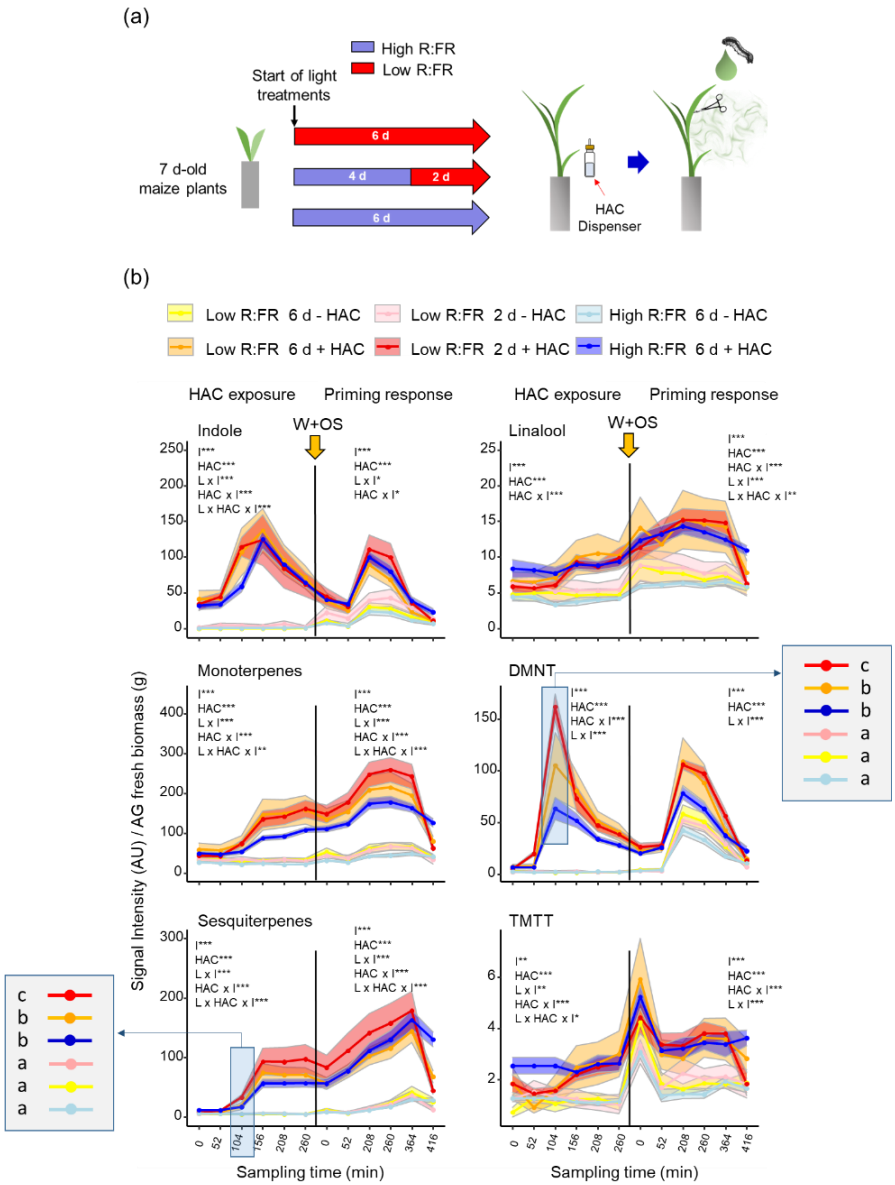


Figure 4. Enhancement of maize volatile responsiveness is specific to short-term exposure to supplemental FR. (a) Schematic overview of the experimental set-up. Seven-day-old W22 maize (*Zea mays*) seedlings were exposed to: (1) high R:FR for 6 days, (2) low R:FR for 6 days, or (3) high R:FR for 4 days 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-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 stands 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.

Supplemental FR enhances JA-Ile accumulation in response to (Z)-3-hexenyl acetate

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 eleven-day old B73 seedlings exposed to low or high R:FR for two days and subsequently to (*Z*)-3-hexenyl acetate and simulated herbivory (Fig. 5).

Exposure to (*Z*)-3-hexenyl acetate directly increased OPDA, JA, JA-Ile, and ABA levels as previously described (Hu *et al.*, 2019) in low and high R:FR-treated plants (Fig. 5a). Light treatments did not affect JA, JA-Ile, and SA levels, but low R:FR alone slightly reduced OPDA concentrations and enhanced ABA levels. In addition, we detected higher levels of JA-Ile, the biologically active jasmonate conjugate, at 45 min after the start of (Z)-3-hexenyl acetate exposure in plants growing under low R:FR.

Upon simulated herbivory, (*Z*)-3-hexenyl acetate pre-treated plants displayed higher OPDA and JA-Ile levels irrespective of the light treatments (Fig. 5b). Irradiation with low R:FR enhanced the levels of JA, JA-Ile, and ABA at 180 min after simulated herbivory irrespective of the volatile pre-treatment (Fig. 5b).

Supplemental FR modulates the expression of volatile biosynthetic genes in response to (Z)-3-hexenyl acetate and herbivory

Next, we tested whether FR-induced emission of volatiles in B73 seedlings correlated with changes in the expression of terpene-related biosynthetic genes during (*Z*)-3-hexenyl acetate exposure or (*Z*)-3-hexenyl acetate exposure followed by simulated herbivory in two time series experiments (Fig. 6).

Exposure to (*Z*)-3-hexenyl acetate 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 and linalool derivatives (Schnee *et al.*, 2002; Xu *et al.*, 2019), was also induced by (*Z*)-3-hexenyl acetate. Expression of *CYP92C5*, involved in the production of DMNT (Richter *et al.*, 2016), was slightly reduced by (*Z*)-3-hexenyl acetate at 180 min after the start of the treatment (Fig. 6a). (*Z*)-3-hexenyl acetate effects were not influenced by light. Low R:FR alone, however, reduced the expression of *CYP92C5* and *FPSS3* genes at 45 min at 90 min, respectively, and slightly enhanced *TPS1* expression at 45 min after the start of the volatile treatment (Fig. 6a).

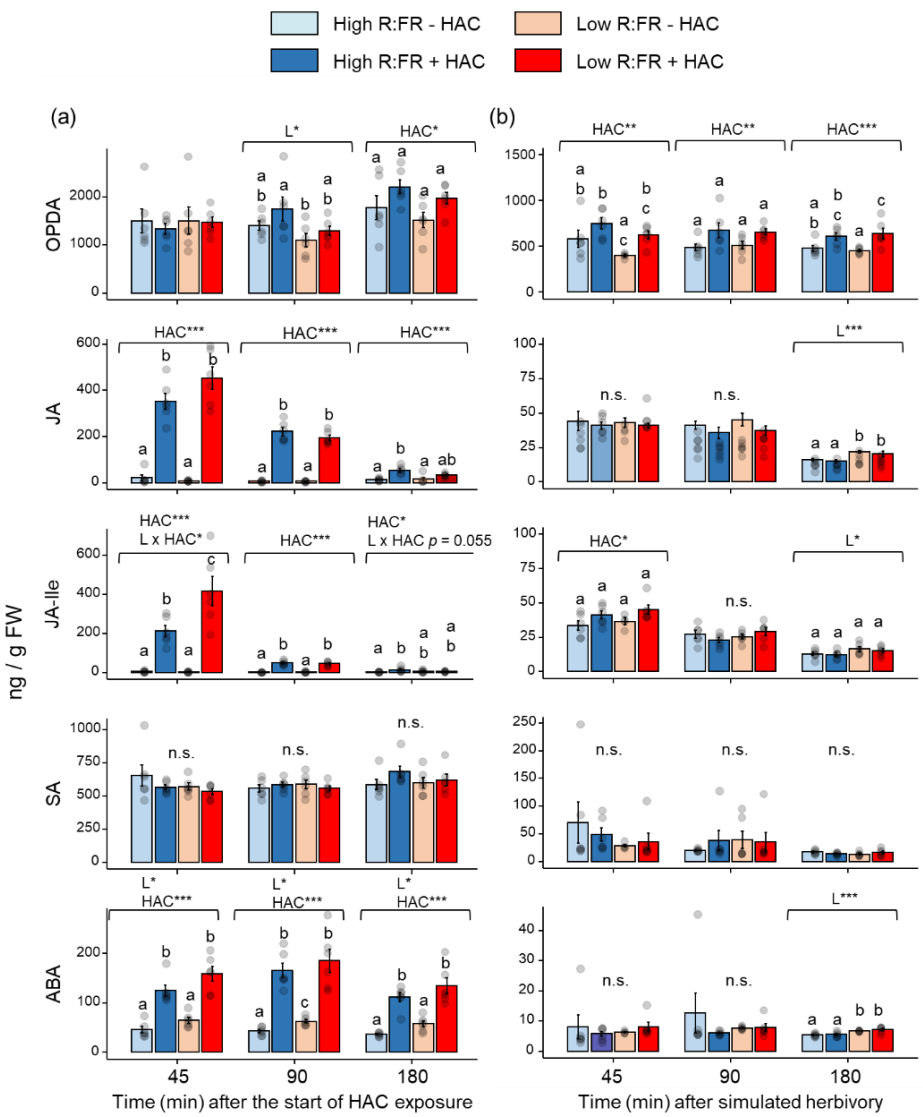


Figure 5. Supplemental FR enhances JA-Ile accumulation in response to (*Z*)-3-hexenyl acetate Concentrations (mean ± SEM, *n* = 4-6) of 12-oxo-phytodienoic acid (OPDA), jasmonic acid (JA), jasmonic acid-isoleucine (JA-Ile), salicylic acid (SA), and abscisic acid (ABA) in high and low R:FR treated B73 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, pre-treatment with (*Z*)-3-hexenyl acetate 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-hexenyl acetate pre-treated plants and led to a stronger (*Z*)-3-hexenyl acetate-mediated CYP92C5 suppression upon simulated herbivory. Irrespective of (*Z*)-3-hexenyl acetate pre-treatment, low R:FR slightly enhanced *TPS2*, *TPS10* and *CYP92C5* expression after simulated herbivory (Fig. 6b). Together, these results

show that low R:FR and (*Z*)-3-hexenyl acetate interact to modulate the expression of terpene biosynthesis genes. However, these slight changes are unlikely to fully explain the differences in volatile emissions.

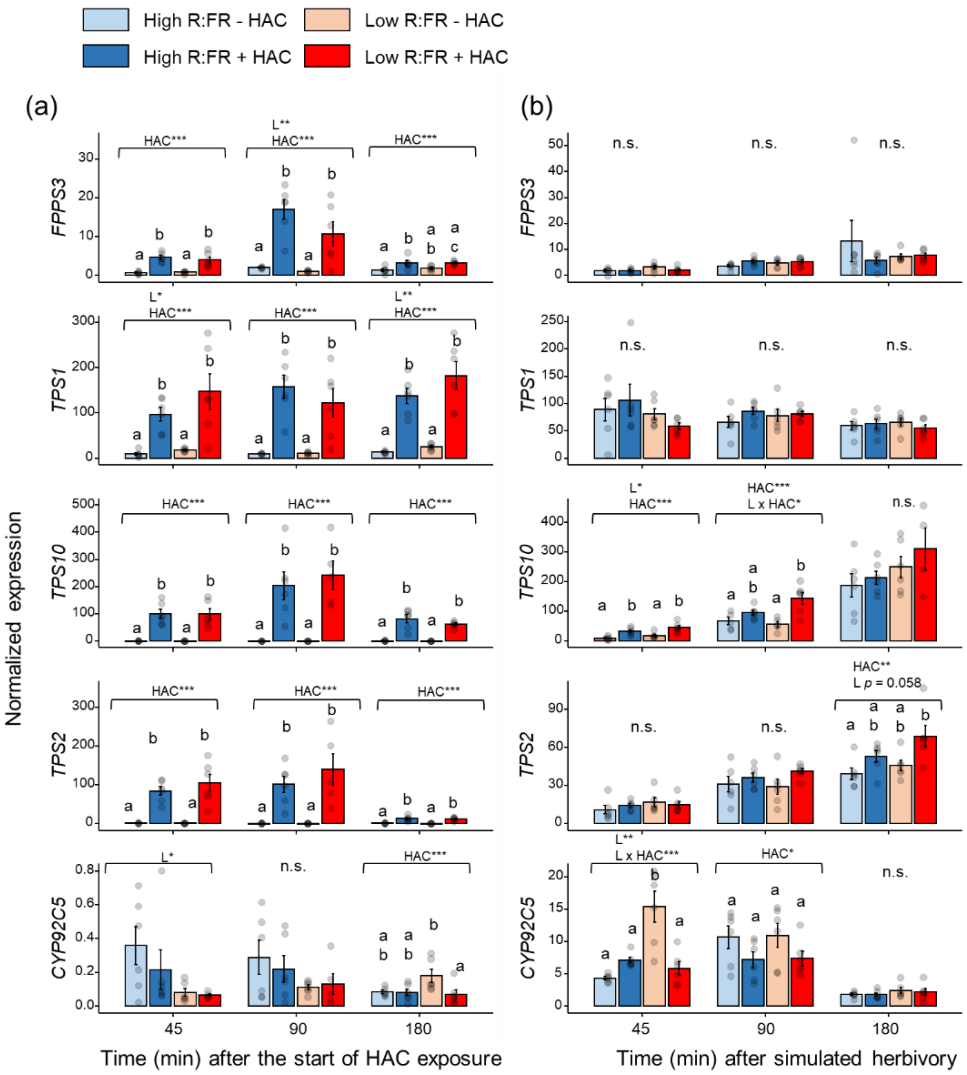


Figure 6. Supplemental FR modulates the expression of volatile biosynthetic genes in response to (*Z*)-3-hexenyl acetate and herbivory. Normalized expression (mean ± SEM, n = 6) of terpene metabolism-related genes determined in aboveground (leaves and stem) tissues of low and high R: FR-irradiated B73 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.*

Supplemental FR increases stomatal conductance and photosynthesis in maize

Emission of sesquiterpenes can be regulated by light-dependent stomata movements in maize (Seidl-Adams *et al.*, 2015). We therefore tested whether differences in stomata conductance could account

for the increase in volatile emission in plants grown under FR-enriched light environments. For this, eleven-day old B73 seedlings were exposed to low or high R:FR for two days and leaf stomatal conductance was determined at 3 h after exposure to (*Z*)-3-hexenyl acetate and at 4 h after subsequent simulated herbivory treatment.

Overall, exposure to (*Z*)-3-hexenyl acetate significantly reduced stomatal conductance (i.e., increased stomatal closure) in maize (Fig. 7ab). Low R:FR, in turn, had a significant positive effect on stomatal conductance (Fig. 7ab), which was not explained by differences in stomatal densities (Supplemental Fig. S9). We did not detect significant interactions between light and (*Z*)-3-hexenyl acetate exposure on stomatal aperture in the absence of herbivory. Upon simulated herbivory, stomatal conductance was reduced close to the wounding sites (Fig. 7cd). In leaf 2, we observed an interaction between treatments upon simulated herbivory, with (*Z*)-3-hexenyl acetate leading to slightly stronger stomatal closure under low R:FR (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-hexenyl acetate had no significant effect on photosynthesis, photosynthetic rates were significantly increased under low R:FR conditions (Fig. 8ab).

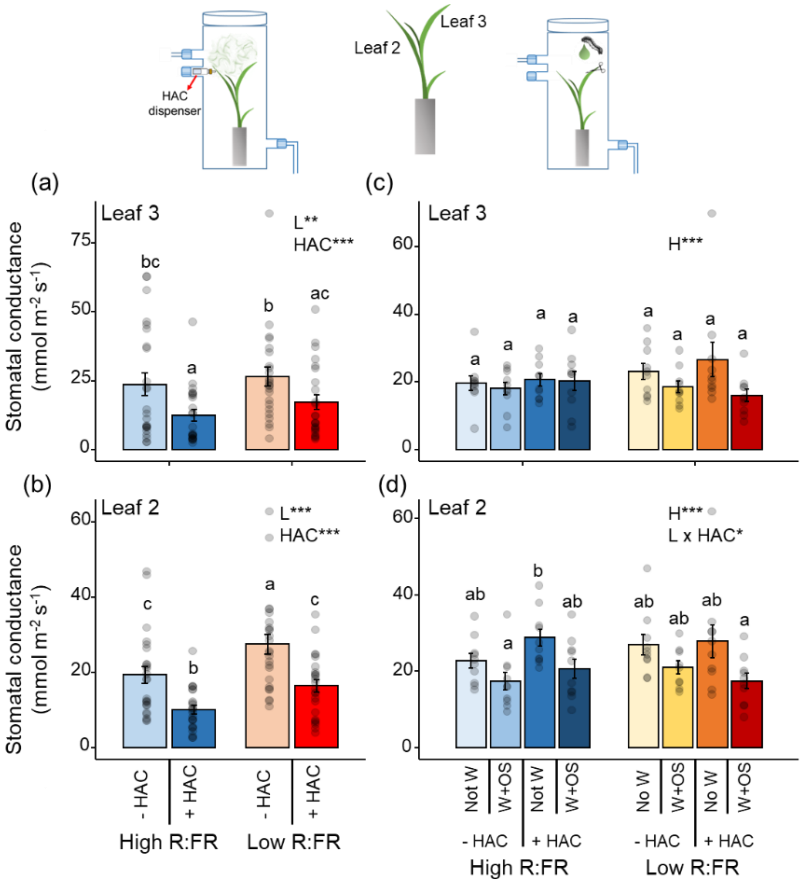


Figure 7. Supplemental FR increases stomatal conductance in maize. Stomatal conductance (mean ± SEM, *n* = 20) determined in the abaxial side of **(a)** leaf #3 and **(b)** leaf #2 from the bottom of low and high R:FR-treated

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).

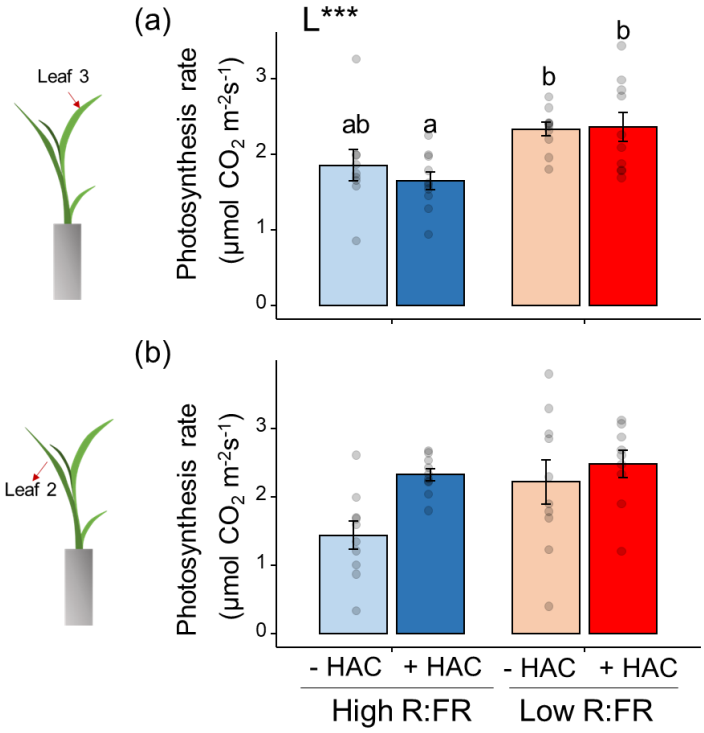


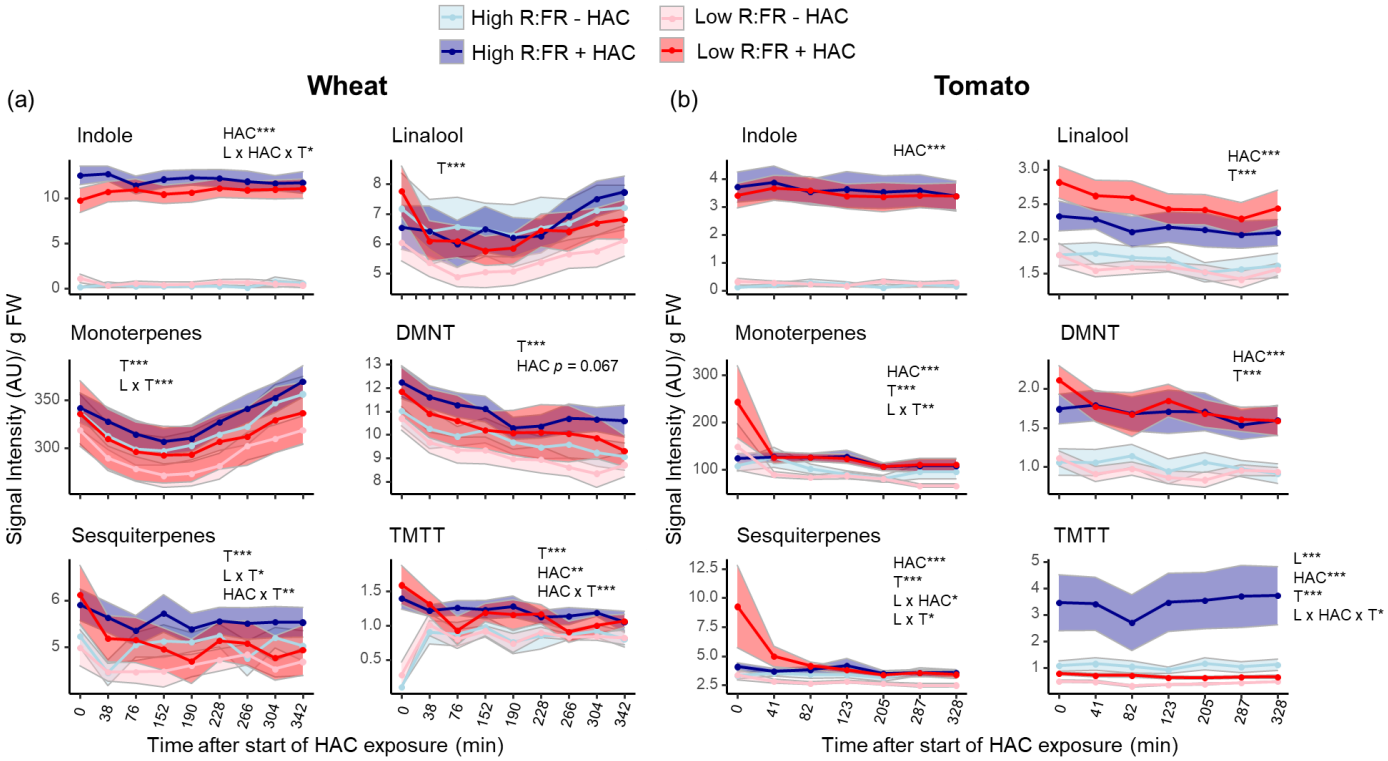
Figure 8. Supplemental FR increases photosynthesis in maize Photosynthesis rate (mean ± SEM, n = 10) determined in the abaxial side of **(a)** leaf 3 and **(b)** leaf 2 from the bottom of high and low R:FR-treated B73 maize plants at 3 h after the start of HAC exposure. Effects of light treatment (L) 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.

Supplemental FR effects on plant responses to (Z)-3-hexenyl acetate are species-dependent

Next, we investigated whether FR-induced volatile emissions in response to (*Z*)-3-hexenyl acetate occurs in two additional plant species, wheat and tomato (Fig. 9). We exposed eighteen-day old wheat plants and four-week-old tomato plants to low or high R:FR for two days and monitored their volatile emissions during (*Z*)-3-hexenyl acetate exposure using the Vocus PTR-TOF-MS system.

In wheat, (*Z*)-3- hexenyl acetate significantly induced the emission of indole, sesquiterpenes, and TMTT (Fig. 9a). Low R:FR reduced the constitutive emissions of monoterpenes and

517 sesquiterpenes and suppressed (*Z*)-3-hexenyl acetate induced emission of indole. In tomato, (*Z*)-3-
518 hexenyl acetate increased the emission of all the volatile signatures (Fig. 9b). Low R:FR reduced the
519 constitutive emission of monoterpenes, sesquiterpenes, and TMTT. Light also affected (*Z*)-3- hexenyl
520 acetate induced responses. Sesquiterpenes emissions were slightly higher at the start of (*Z*)-3-
521 hexenyl acetate exposure in low R:FR-treated tomato plants, while induced TMTT emissions were
522 strongly suppressed. This suggests that low R:FR-induced volatile emission in response to (*Z*)-3-
523 hexenyl acetate is species-dependent.



524

525 **Figure 9. Supplemental FR effects on plant responses to (*Z*)-3-hexenyl acetate are species-dependent.**
526 Time-series analysis of volatile emissions (mean ± SEM, n = 6) determined in low and high R:FR-treated **(a)**
527 wheat (*Triticum aestivum*) and **(b)** tomato (*Solanum lycopersicum*) plants during exposure to (*Z*)-3-hexenyl
528 acetate (+ HAC) or clean air (- HAC). HAC exposure and volatile measurements started between 9:00 and 10:00
529 am. The effects of the sampling time (T), HAC, light treatment (L) and their interactions on volatile emissions
530 were tested separately for the HAC exposure and priming phase using linear mixed-effects models. Statistically
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532 the homoterpenes (E)-4,8-dimethyl-1,3,7-nonatriene and (E, E)-4,8,12-trimethyltrideca-1,3,7,11-tetraene,
533 respectively. AU refers to arbitrary units.

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Discussion

In dense canopies, plants can detect the presence and physiological state of neighboring plants through light and volatile cues. Whether plants can integrate these two environmental cues into specific responses, however, have been unclear so far. Our results demonstrate that plants indeed combine both into distinct defense responses and volatile signaling patterns. Below, we discuss the potential mechanisms and ecological consequences of this phenomenon in the context of plant-plant and plant-herbivore interactions.

Herbivore induced plant volatiles have been shown to 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). We find that direct and priming responses to herbivore-induced plant volatiles are enhanced in plants exposed to low R:FR ratios, a central light cue that indicates the presence of neighbors and vegetative shade. The enhanced responses reported here included a stronger accumulation of the bioactive jasmonate JA-Ile, slightly higher expression of volatile biosynthesis-related genes, and a markedly higher release of induced volatiles. Thus, overall, FR-enriched light environment prompts maize plants to respond more strongly to herbivory-induced volatiles emitted by their neighbors. Synergistic effects have been observed for different environmental cues to boosts physiological responses in plants (Gouinguené & Turlings, 2002; Wit *et al.*, 2016).The findings presented here therefore show that plants can also integrate herbivory-induced volatiles and light competition cues from neighboring plants, which expands the known repertoire and sophistication of plant perception and plant-plant interactions.

Plant responses to volatiles are regulated by the jasmonate defense signaling (Arimura *et al.*, 2000; Hu, 2022). The jasmonate pathway is part of an integrated signaling network that controls plant growth and defense responses, including the emission of volatiles (Schmelz *et al.*, 2003; Bosch *et al.*, 2014; Mujiono *et al.*, 2021). In maize, (Z)-3-hexenyl acetate exposure can induce the accumulation of jasmonates and the expression of JA defense marker genes before and after herbivory induction (Hu *et al.*, 2019). Here, we confirmed that (*Z*)-3-hexenyl acetate strongly up-regulated JA signaling in maize. Furthermore, we found that irradiation with low R:FR 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 signaling in dicotyledonous species (Chico *et al.*, 2014; Leone *et al.*, 2014; Fernández-Milmanda *et al.*, 2020). Yet, low R:FR induced characteristic shade-associated morphological responses in maize, such as the elongation of stems. Furthermore, we observed a strong induction 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 was 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 will have to be conducted to reveal the importance of the enhanced jasmonate response and the potential role of auxin and ABA in maize responses to (*Z*)-3-hexenyl acetate.

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-hexenyl acetate 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. 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-hexenyl acetate 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-hexenyl acetate induction of volatile biosynthesis quickly increased their concentration in the intercellular air spaces, enhancing volatiles diffusion gradients and overruling stomatal limitations.

Interestingly, low R:FR 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 corn, 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 are in line with the expected stomatal and photosynthetic

responses of maize plants in dense canopies characterized by a reduced PAR and FR-enriched light environment. 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. On the other hand, 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 is the cause or the result of increased photosynthetic rates and/or of inhibition of ABA signaling needs to be established. Yet, it is conceivable that the effects of supplemental FR on the emission of volatiles in response to (*Z*)-3-hexenyl acetate 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).

Plants perceive neighbor-associated changes in light quality and quantity through photoreceptors, of which the phyB is the predominant sensor of R and FR light signals (Pierik & Wit, 2014). Like the *phyB* mutant in Arabidopsis (Ballaré, 2009), the *phyB1phyB2* maize double mutant displayed constitutive shade avoidance syndrome (SAS), supporting the role of phyB in regulating SAS in maize. Surprisingly, and in contrast to our low R:FR treatments, we found that *phyB1phyB2* plants displayed an overall diminished response to (*Z*)-3-hexenyl acetate. These results are in line 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 result in different effects than short term inactivation via changes in R:FR light cues. Indeed, longer exposure of maize seedlings to low R:FR resulted in a weaker boost of low R:FR on (*Z*)-3-hexenyl acetate induced volatile emissions. At the same time, the *phyB1phyB2* mutant retained some of its ability to respond to low R:FR with higher (*Z*)-3-hexenyl acetate induced volatile emissions, which reinforces the potential effects of supplemental FR on the photosynthetic machinery. We conclude that (i) the duration of phyB inactivation is a determinant factor for light and volatile cue integration, and that (ii) alternative mechanisms, including an enhanced photosynthetic efficiency and/or other FR photoreceptors, may 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). PhyA therefore represents a candidate that may be involved in the integration of 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 capacity to attract natural enemies in a setting when their recruitment is a competitive struggle (Kessler & Baldwin, 2001; Aartsma *et al.*, 2017). The volatiles may also decrease ovipositing of gravid female moths (Moraes *et al.*, 2001). However, the situation is complicated by the fact that 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 and tri-trophic interaction dynamics, and community structure.

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Author contributions

ME conceived the study. ME, RE-B, BCJS and CAMR designed experiments. RE-B performed experiments. 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.

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