Volatiles from low R:FR-treated maize plants increase the emission of herbivore-induced plant volatiles in their neighbors

Rocío Escobar-Bravo[1,2] *, Bernardus C.J. Schimmel[1,3], Matthias Erb[1]

[1]Institute of Plant Sciences, University of Bern, 3013, Bern, Switzerland.

[2]Current address: Enza Zaden, Haling 1-E,1602 DB, Enkhuizen, The Netherlands

[3]Current address: Institute of Environmental Biology, Utrecht University, Padualaan 8, 3584 CH 14 Utrecht, The Netherlands

***Author for correspondence:** Rocío Escobar-Bravo (rocioebe@hotmail.com)

ABSTRACT

Low Red (R) to Far Red (FR) light ratios, a light signal associated with vegetation shade, can prompt intact maize (*Zea mays*) plants to constitutively emit more volatiles when exposed to herbivory-induced plant volatiles (HIPVs). Here we investigated how simulated shading affects priming responses in the context of volatile-mediated plant-plant interactions. Receiver maize plants were exposed to either constitutive volatile organic compounds (cVOCs) or HIPVs from emitter maize plants, while we manipulated R:FR light conditions of receivers or emitters. Priming responses in the receivers were then assessed by measuring real-time volatile emissions following simulated herbivory. We show that low R:FR light enhances HIPVs emissions in plants previously exposed to HIPVs from neighbours independently of the light conditions of emitters. We also demonstrate that both cVOCs and HIPVs emitted by maize grown under low R:FR amplify HIPVs emissions in their neighbours. This amplified response could not be explained by FR-mediated changes in the release of green leaf volatiles or terpenoids by emitters, thus suggesting the involvement of other VOCs. We conclude that volatile-mediated plant-plant interactions can be expected to become more intense in denser canopies due to light-mediated amplification of volatile emission and responsiveness.

Key words

Far-red light, herbivores, maize, plant defenses, priming, volatiles

INTRODUCTION

Plants can sense the presence of neighbors through light, chemical, and mechanical cues (Huber et al., 2020; Ninkovic et al., 2021; Pantazopoulou et al., 2023). This ability to detect nearby plants helps them to assess their competitive environment, including the availability of light and soil nutrients, as well as the health and potential threats posed by pests and pathogens. By integrating these signals, plants can strategically allocate their resources towards growth or defense, thus enhancing their chances of survival.

Neighbor detection through light cues occurs via the photoreceptor-mediated perception of light spectral changes in the canopy. Blue (λ =400-500 nm) and red (R) (λ = 600-700 nm) light wavelengths are preferentially absorbed by photosynthetic pigments of plant leaves while far red (FR) light is mainly reflected or transmitted. In dense canopies, this results in a reduction in R:FR ratios, a light cue that can promote upward bending of the leaves (hyponasty) and stem elongation (Pierik and Ballaré 2021). These photomorphogenic changes help the plant to reach more illuminated areas in the canopy and outcompete their neighbors (Pierik and Ballaré 2021) In addition, FR photons can support growth by promoting photosynthesis in dicotyledonous and C3 and C4 monocotyledonous species, including maize (*Zea mays*), when added to a background of white light (Zhen and Bugbee 2020; Escobar-Bravo et al. 2024; Huber et al. 2024).

Beyond light, plants also perceive their environment through volatile organic compounds (VOCs) (Escobar-Bravo, Lin & Waterman, 2023). These compounds include volatile hormones (e.g., ethylene, methyl salicylate, and methyl jasmonate), terpenoids, benzenoids (e.g., indole), phenylpropanoids, and fatty acid-derived molecules such as green leaf volatiles (GLVs). Plant VOCs can be constitutively emitted and modulated by the plant interactions with the environment. Changes in their emission therefore reflect the plant status and the abiotic and/or biotic stresses it encounters. Upon herbivory, for instance, plants can emit herbivory-induced plant volatiles (HIPVs) that repel the attacker, attract natural predators of the herbivore, and/or alter the defense status in non-attacked neighbors (Turlings and Erb 2018; Hu et al. 2021).

Some HIPVs, such as the green leaf volatiles (*Z*)-3-hexenal, (Z)-3-hexen-1-ol, or (Z)-3-hexenyl acetate, can directly induce the emission of volatiles in intact plants and prime their volatile emissions (Farag and Pare 2002; Engelberth et al. 2004; Hu et al. 2019; Escobar-Bravo et al. 2024). Similarly, other HIPVs like indole, the monoterpene β-ocimene, and the homoterpene (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) can induce or prime plant defenses (Erb et al. 2015; Jing et al. 2021; Onosato et al. 2022). Defense priming can confer ecological benefits to plants, as the exposure to the priming stimulus prepares them to display a faster, stronger, and more lasting defense response when the actual herbivore or pathogen attack takes place (Conrath et al. 2015; Martinez-Medina et al. 2016). The mechanisms of volatile-mediated defense priming downstream of volatile perception have been amply characterized. Once HIPVs get into plant tissues, they induce the expression of biotic and abiotic stress-responsive genes in a Ca^{2+} dependent manner, including the activation of the jasmonic acid (JA) defense signaling pathway that controls the emission of HIPVs (Zebelo et al. 2012; Christensen et al. 2013; Engelberth et al. 2004; Hu et al. 2019; Ye et al. 2019; Jing et al. 2021; Aratani et al. 2023; Aguirre et al. 2023; Wang et al. 2023).

How VOC-mediated transfer of information between plants occurs in dense canopies is not well known. The FR-enriched light environment within vegetation canopies might play a crucial role in these interactions by altering (1) the emitter's volatile emissions, (2) the ability of the receiver to perceive and respond to the volatile cue/s, or (3) both. For instance, Kegge et al. (2015) showed that barley (*Hordeum vulgare*) emits different volatiles under low R:FR ratios, influencing the carbon allocation in neighboring plants. Aguirre et al. (2023) reported that maize exposure to (*Z*)-3-hexenyl acetate in the dark does not prime plant defenses, suggesting that light-mediated induction of stomatal opening is required for signal integration of (*Z*)-3-hexenyl acetate in receiver plants. More recently, we have demonstrated that low R:FR light conditions can change the perception of HIPVs in maize plants (Escobar-Bravo et al. 2024). Our study showed that FR-light enrichment prompts intact maize plants to emit more volatiles when exposed to HIPVs from *Spodoptera littoralis*- infested neighbors or to *Z*-3-hexenyl acetate alone. We also showed that low R:FR-treated plants pre-exposed to *Z*-3-hexenyl acetate displayed stronger HIPVs emissions after simulated herbivory and, therefore, a stronger priming response. Yet, whether low R:FR-induced priming response also occur in plants exposed the whole bouquet of HIPVs from herbivore-infested neighbors was not determined. Furthermore, whether FR light enrichment can alter volatile-mediated interactions between maize plants by inducing changes in the VOCs emissions of emitter plants is unknown.

Here, we have investigated whether low R:FR affects HIPVs-induced priming responses in maize by altering (1) the volatile perception of the receivers, and/or (2) the volatile emissions of the emitters. For this, we first exposed plants to low R:FR ratios as a light cue for vegetation shade or high R:FR ratios as control light conditions, and we determined volatile emissions in simulated herbivory-induced plants that were previously exposed to constitutive volatile organic compounds (VOCs) or HIPVs emissions from intact or *S. littoralis*-infested plants, respectively. Next, we conducted a multifactorial experiment where we tested whether HIPVs emitted by intact or simulated herbivory-induced maize plants growing under low or high R:FR conditions trigger different defense priming responses in the receivers, and whether these differences could be explained by changes in the GLVs emissions of the emitters.

MATERIALS AND METHODS

Plants

Experiments were conducted with the maize (*Zea mays*) inbred line B73. 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. 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.

112 **Light treatments**

113 Light treatments were conducted in a custom-made high-throughput phenotyping platform designed for
114 volatile analyses previously described in Escobar-Bravo et al. (2024). Eleven-day old maize seedlings
115 were exposed to low (~0.5) or high (~2.65) R:FR ratios (R, λ = 600-700 nm; FR, λ = 700-800 nm)
116 by providing supplemental FR to the same white light background (120 ± 15 μmol m^{-2} s^{-1})
117 (Supplemental Fig. S1) with a photoperiod of 16/8 h light/dark. This low R:FR corresponds to the
118 ratios observed in high density maize canopies (Maddonni et al. 2002) where low PAR levels as
119 the ones used in our experimental set-up are commonly observed (Xue et al. 2016). Light
120 treatments were separated by white opaque curtains and started between 9:00 and 10:00 AM two
121 days before the plant-plant interaction experiments.

122 **Plant-plant interaction experiments**

123 In the first experiment (Supplemental Fig. S2), we exposed emitter plants to low or high R:FR light
124 conditions and the emitters to low R:FR light conditions. After two days, low R:FR-treated emitter plants
125 were infested with ten second-instar *S. littoralis* larvae or left uninfested. Right after infestation, low and
126 high R:FR-treated receiver plants were exposed to volatiles emitted by *S. littoralis*-infested or non-
127 infested low R:FR treated plants. The chambers of emitter and receiver plants were connected using
128 Teflon tubing. Clen air flow moved from the emitter's chamber towards the receiver's chamber. After 20
129 h, the Teflon tubing connecting emitters and receivers' chambers was removed, and receiver plants
130 were induced with simulated herbivory. Receiver's volatile emissions were then recorded in time-series
131 analysis using a PTR-TOF-MS system. In the second experiment (Supplemental Fig. S3), we employed
132 the same set-up, but emitters were exposed to high R:FR light conditions.

133 In the third experiment (Supplemental Fig. S4), we exposed both emitter and receiver plants to
134 high or low R:FR conditions for 2 days. Next, emitter and receivers' chambers were connected via Teflon
135 tubing as in previous experiments and the emitter plants immediately induced with simulated herbivory
136 or left intact. After 6 h, the Teflon tubing connecting emitters and receivers' chambers was removed,
137 and all the receiver plants were induced with simulated herbivory. Receiver's volatile emissions were
138 then recorded in time-series analysis using a PTR-TOF-MS system. This experiment was repeated
139 three times using three replicates per combined treatment each time. Data from the three repetitions
140 were pooled prior to the statistical analysis.

141 **PTR-TOF-MS volatile emission analysis**

142 Plant volatile emission was determined using the automated high-throughput real-time phenotyping
143 platform and the methods described in Escobar-Bravo et al. (2024)

144 **Simulated herbivory**

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

149 **Statistical analysis**

150 Data analysis was conducted in R version 4.0.4 (R core Team, 2016). Effects of (1) sampling time, (2)
151 light receiver, (3) light emitter, (4) volatiles from the emitter, and (5) their interactions on volatile
152 emissions in the different experiments were tested in linear mixed-effects models (LME) using nlme
153 library. Individual plants were included in the model as a random factor and a correlation structure when
154 autocorrelation among residuals was found to be significant ($p < 0.05$). Fitted models were subjected
155 to type III analyses of variance (ANOVAs) to produce a summary of the F- and p statistics (car package)
156 (Fox et al. 2012). When needed, data from volatiles signatures were log10 or squared root transformed
157 prior analysis to correct for heteroscedasticity.

158 **RESULTS**

159 **Low R:FR light enhances HIPVs-mediated priming of volatile emissions in maize**

160 Low R:FR light ratios can increase the emission of volatiles in intact maize plants when these are
161 exposed to HIPVs emitted by low R:FR-treated neighbors (Escobar-Bravo et al. 2024). To determine
162 whether low R:FR and pre-exposure to HIPVs emitted by herbivore-infested neighbors affects
163 subsequent maize responses to herbivory, we conducted a series of plant-plant interactions
164 experiments.

165 In the first experiment, we exposed 11-day old B73 maize seedlings to low or high R:FR light
166 conditions for two days and measured their volatile emissions after exposure to volatiles emitted by *S.*
167 *littoralis*-infested or non-infested low R:FR treated plants followed by induction with wounding and the
168 application of *S. littoralis* oral secretions (Fig. S2 and Fig. 1ab). Upon simulated herbivory, emission of
169 all the volatile signatures increased over time (Fig. 1b). This induction was stronger under low R:FR
170 irrespective of the pre-exposure to emitters' volatiles. Emission of monoterpenes, sesquiterpenes,
171 DMNT, and TMTT were higher in low R:FR treated plants compared to high R:FR treated plants, except
172 for indole. Light also influenced maize responses to volatiles. The emissions of sesquiterpenes,
173 monoterpenes, TMTT, and DMNT were higher in low R:FR-treated plants exposed to HIPVs from
174 herbivore-infested plants, whereas no induction, or even the opposite pattern, was observed in high
175 R:FR-treated plants.

176 In the second experiment, we used the same set-up, but plants were instead exposed to
177 volatiles emitted by high R:FR treated plants that were either infested with *S. littoralis* larvae or non-
178 infested (Fig S3, and Fig. 2). Emission of all the volatiles increased over time upon simulated herbivory
179 in low and high R:FR treated plants. Unlike the first experiment, plants exposed to low R:FR and
180 volatiles from high R:FR treated plants overall emitted less indole, monoterpenes, DMNT and TMTT
181 than plants exposed to high R:FR and the same volatiles (Fig. 2). Prior exposure to volatiles from high
182 R:FR treated plants infested with *S. littoralis* larvae enhanced the emission of monoterpenes,
183 sesquiterpenes, and linalool (albeit to lesser extent) in low R:FR-treated plants upon simulated
184 herbivory. The opposite was observed for plants irradiated with high R:FR ratios, where prior exposure
185 to HIPVs slightly decreased the emission of monoterpenes, sesquiterpenes, linalool and DMNT, and
186 the opposite was observed for indole.

These results suggest that low R:FR can enhance plant responses to HIPVs emitted by both high and low R:FR-treated plants, but this induction seems to be stronger when plants were exposed to volatiles emitted by neighbors growing under low R:FR ratios.

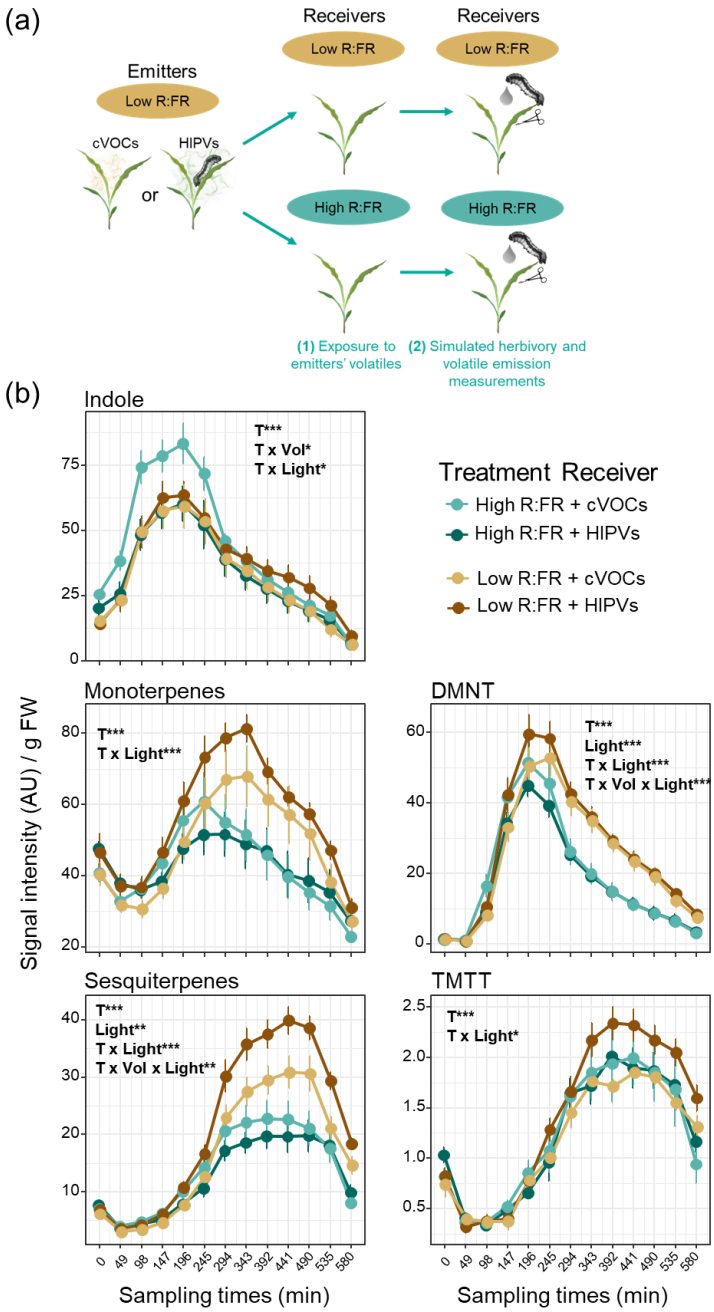
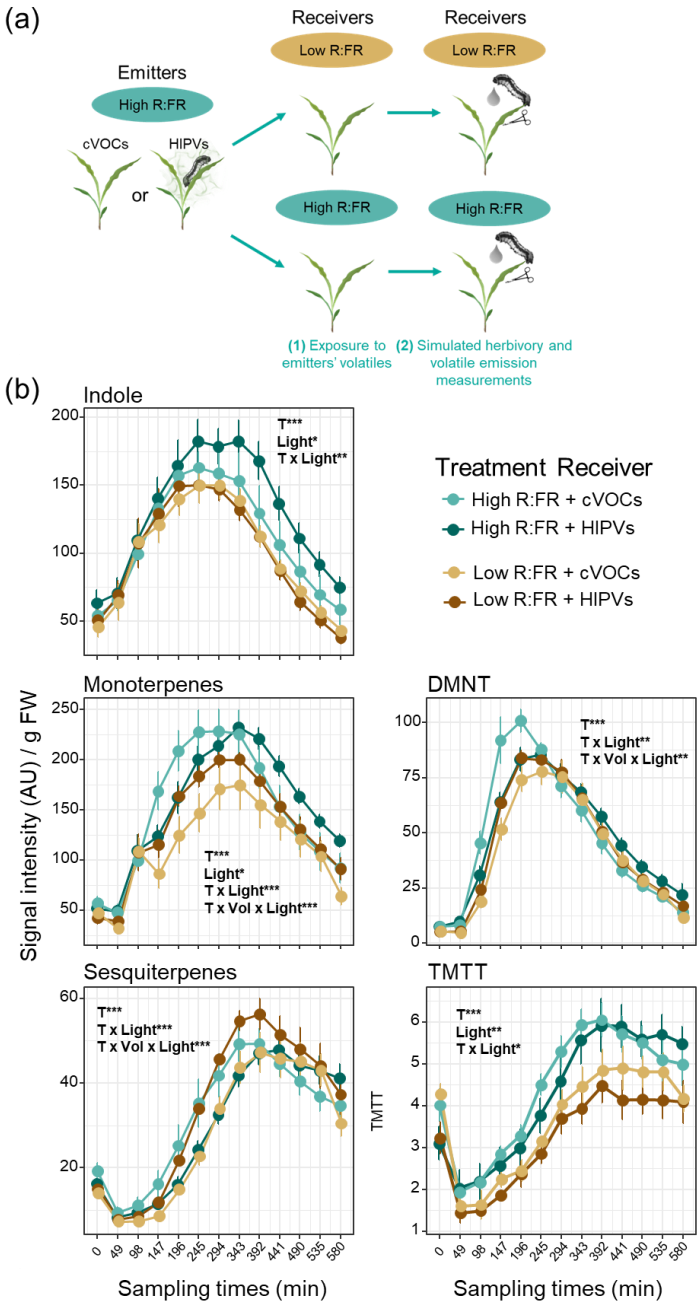


Figure 1. Low R:FR enhances priming of monoterpenes, sesquiterpenes and TMTT emissions in maize plants pre-exposed to HIPVs from low R:FR treated plants. (a) Schematic overview of the emitters and receivers' treatments. (b) Time-series analysis of volatile emissions (mean ± SEM, n = 6) collected from receiver plants pre-exposed to cVOCs or HIPVs from low R:FR-treated plants during 20 h and followed by simulated herbivory (wounding + oral secretions from *S. littoralis*). The effects of sampling time (T), light treatment of receivers (Light), volatiles from emitters (Vol), and their interactions on receivers' 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-

198 1,3,7-nonatriene and (*E*, *E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene, respectively. AU refers to arbitrary
199 units.



200 **Figure 2. Low R:FR enhances priming of monoterpenes and sesquiterpenes emissions in maize plants pre-**
201 **exposed to HIPVs from high R:FR treated plants.** (a) Schematic overview of the emitters and receivers'
202 treatments. (b) Time-series analysis of volatile emissions (mean ± SEM, n = 6) collected from receiver plants
203 pre-exposed to cVOCs or HIPVs from low R:FR-treated plants during 20 h and followed by simulated
204 herbivory (wounding + oral secretions from *S. littoralis*). The effects of sampling time (T), light treatment of
205 receivers (Light), volatiles from emitters (Vol), and their interactions on receivers' volatile emissions were
206 tested using linear mixed-effects models. Statistically significant effects are shown in each graph (*p < 0.05,
207 ** p < 0.001, *** p < 0.001). DMNT and TMTT stand for the homoterpenes (*E*)-4,8-dimethyl-1,3,7-nonatriene
208 and (*E*, *E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene, respectively. AU refers to arbitrary units.

209

Volatiles emitted by low R:FR treated maize plants increase the emission of herbivore-induced plant volatiles in receiver plants

To test whether maize plants respond differently to volatiles emitted by plants under low R:FR ratios (i.e., simulated shade), we conducted a factorial experiment where high and low R:FR-treated plants were exposed to volatiles emitted by low or high R:FR treated plants that were either induced with simulated herbivory or left intact (Supplemental Fig. S4). After 6 h of exposure to constitutive or herbivory-induced volatiles from emitters, receiver plants were induced with simulated herbivory and their volatile emissions were determined by PTR-TOF-MS in time series analyses. For the sake of clarity, we present the results of this experiment in two panels grouped by the light treatment of the receiver (Fig. 3).

Maize plants exposed to low R:FR responded differently to volatiles emitted by low and high R:FR-treated plants (Fig. 3a). Upon simulated herbivory, monoterpenes, sesquiterpenes and linalool emissions were higher when low R:FR-treated plants were exposed to volatiles emitted by low R:FR-treated plants than by emitter plants irradiated with high R:FR (Fig. 3a). This induction was statistically significant for sesquiterpenes, and it was independent of the herbivory treatment of the emitters. In addition, indole emissions were increased in low R:FR-treated plants when they were pre-exposed to volatiles from not-wounded low R:FR-treated plants.

Receiver plants exposed to high R:FR ratios and volatiles emitted by low R:FR-treated plants showed a slight increase in the emission of monoterpenes and sesquiterpenes, but this was not statistically significant for any of the volatile signatures (Fig. 3b).

Further analysis on the sum of all the volatiles emitted over the sampling period in both data sets showed that (1) low R:FR-treated plants overall emitted more volatiles after exposure to volatiles from emitters and subsequent simulated herbivory and (2) volatiles emitted by intact (cVOCs) and herbivory-induced (HIPVs) low R:FR-treated plants enhance the emission of HIPVs in neighboring plants (Fig. 4).

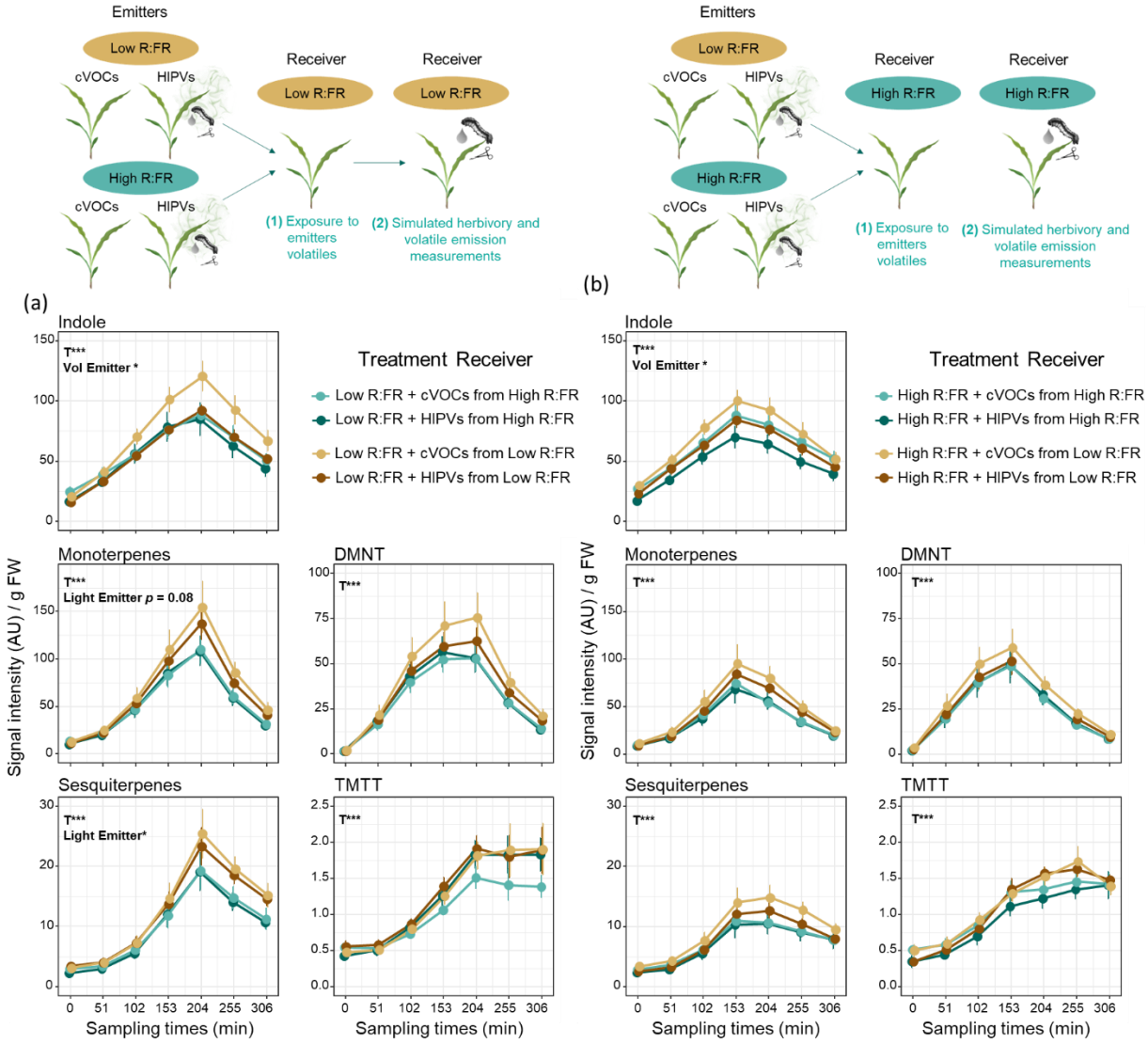


Figure 3. Volatiles emitted by low R:FR-treated plants enhance emissions of HIPVs in low R:FR-treated plants. Time-series analysis of volatile emissions (mean ± SEM, n = 9) collected from **(a)** low R:FR-treated or **(b)** high R:FR-treated receiver plants pre-exposed to cVOCs or HIPVs from low or high R:FR-treated plants during 6 h and followed by simulated herbivory (wounding + oral secretions from *S. littoralis*). Pooled data from three independent experiments are shown. The effects of sampling time (T), light treatment of emitters (Light Emitter), volatiles from emitters (Vol Emitter), and their interactions on receivers' 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.

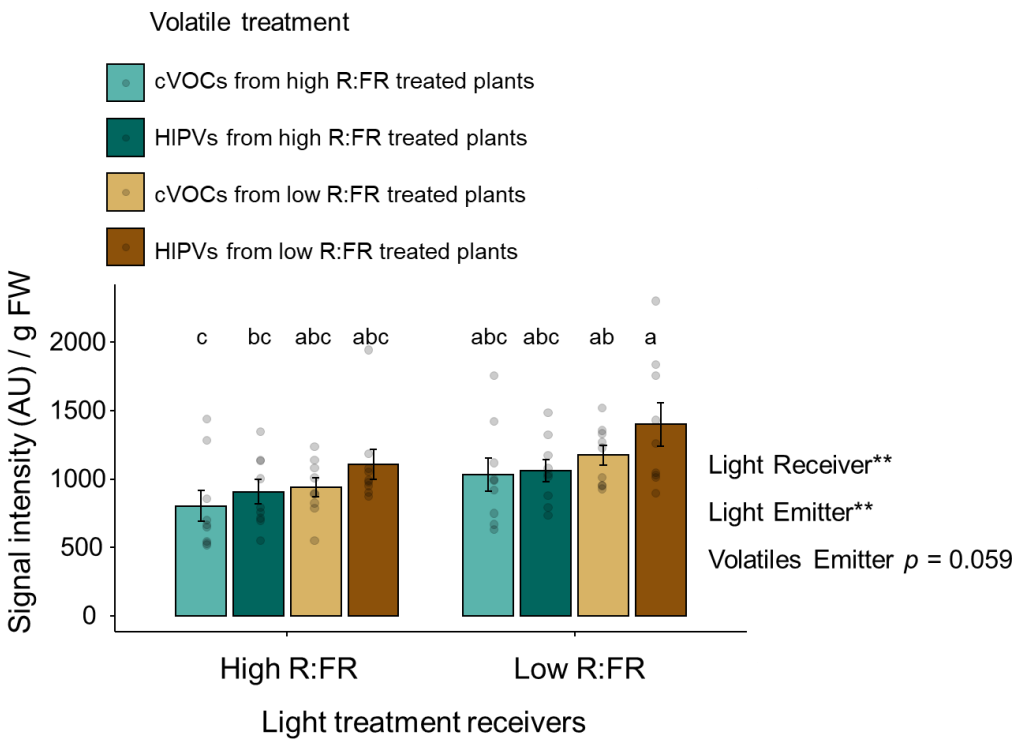


Figure 4. Sum (mean ± SEM, n = 9) of the emission of all the volatiles signatures (indole, monoterpenes, sesquiterpenes, DMNT, and TMTT) measured over a period of 5 h upon simulated herbivory induction in receiver plants. Low or high R:FR-treated receiver plants were pre-exposed to cVOCs or HIPVs from low or high R:FR-treated plants during 6 h followed by simulated herbivory (wounding + oral secretions from *S. littoralis*).The effects of light treatment of the receiver (Light Receiver), light treatment of the emitter (Light Emitter), the volatiles from the emitters (Volatiles Emitter), and their interactions on receivers' volatile emissions were tested using three-way ANOVA followed by Tukey-HSD post hoc test. Statistically significant effects are shown in the graph (** $p < 0.001$). Different letters denote significant differences among groups at $p < 0.05$.

Low R:FR slightly increases (*Z*)-3-HAL emission but not (*Z*)-3-HOL, and (*Z*)-3-HAC

Green leaf volatiles such as (*Z*)-3-hexenal, (*Z*)-3-hexenol, and (*Z*)-3-hexenyl acetate are HIPVs emitted by maize that can primed defenses in neighboring plants (Farag and Pare 2002; Engelberth et al. 2004). To determine whether FR light increases the emission of these volatiles, we compared the GLVs emissions in simulated herbivory-induced and unwounded plants that were previously exposed to low or high R:FR conditions for 2 days (Fig. 5).

Simulated herbivory significantly induced the emissions of (*Z*)-3-HAL, (*Z*)-3-HOL, and (*Z*)-3-HAC in both low and high R:FR treated plants (Fig. 5). Low R:FR did not significantly affect (*Z*)-3-HOL, and (*Z*)-3-HAC emissions in unwounded and simulated herbivory-induced plants (Fig. 5bc). (*Z*)-3-HAL emission levels were higher in low R:FR-treated plants compared to high R:FR treated plants (Fig. 5a).

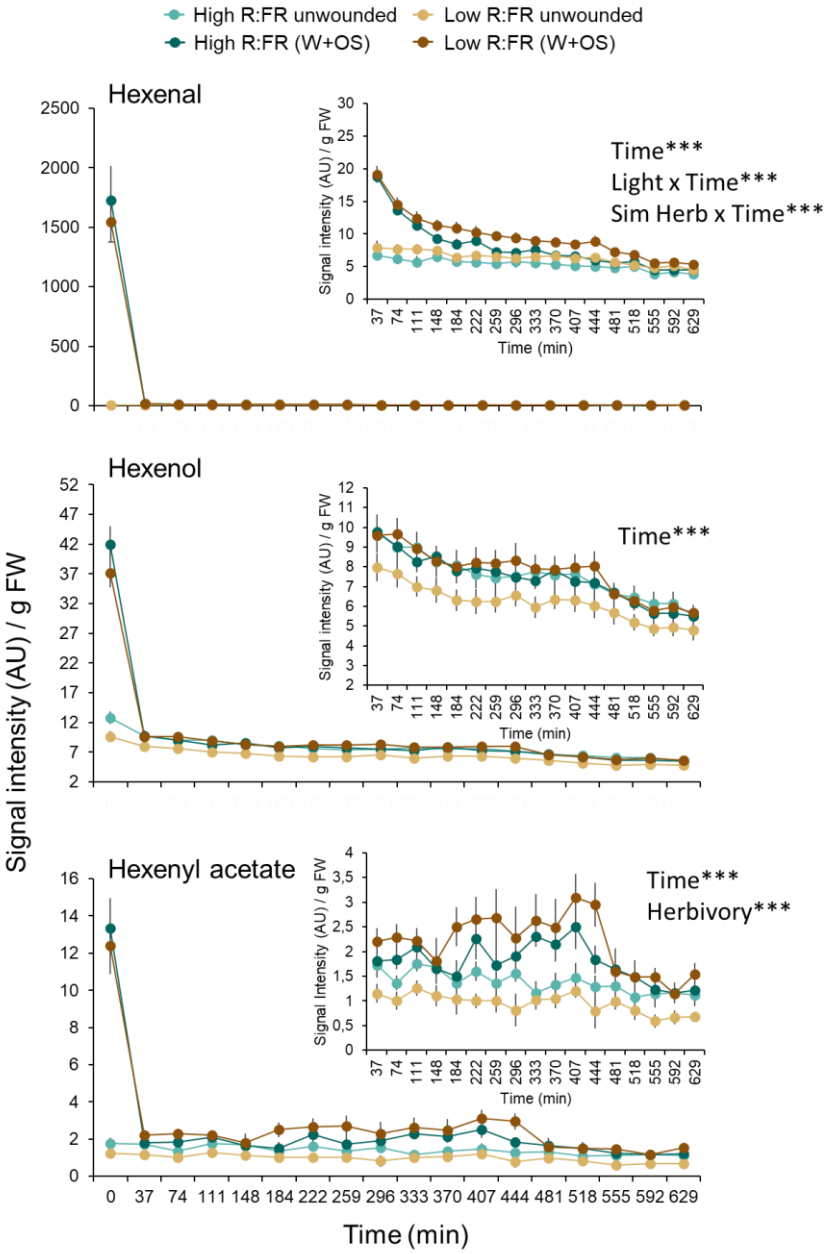


Figure 5. Time series analysis of green leaf volatile emissions (mean ± SEM, *n* = 8) determined by PTR-TOF-MS at intervals of 37 min in high and low R:FR-treated B73 maize (*Zea mays*) plants after simulated herbivory. Volatile measurements started at 12:30 pm, 10 min after simulated herbivory treatment (wounding and application of *Spodoptera littoralis* oral secretions; W+OS). The effects of sampling time (Time), light treatment (Light), simulated herbivory (Herbivory) and their interactions on volatile emission were tested using linear mixed-effects models. Plant unit was included as the random intercept as well as a correlation structure when autocorrelation among residuals was found significant ($p < 0.05$). Statistically significant effects are indicated in each graph. *** $p < 0.001$.

291 **DISCUSSION**

292 Our study shows that maize plants growing under FR-enriched light conditions (low R:FR ratios) not
293 only display a higher emission of HIPVs after exposure to volatile cues from neighbors, but they also
294 emit volatiles that increase the emission of HIPVs in neighboring plants. These findings contribute to
295 unraveling the complexity of volatile-mediated interplay between plants in vegetation canopies where
296 shade-associated light cues might play a key role.

297 Our previous study demonstrated that low R:FR enhances HIPVs emissions in plants exposed
298 to (*Z*)-3-hexenyl acetate alone followed by simulated herbivory (Escobar-Bravo et al., 2024). Here we
299 show that maize plants exposed to low R:FR also display a stronger priming response upon pre-
300 exposure to the HIPVs emitted by herbivore-infested plants. We hypothesize that this response might
301 be partially explained by FR-mediated positive effects on stomata opening and photosynthesis (Zhen
302 and Bugbee 2020; Escobar-Bravo et al. 2024; Huber et al. 2024). As stomata are the entry ports of
303 VOCs into intact plants (Aratani et al. 2023) and modulate indole and (*Z*)-3-HAL-mediated priming
304 responses in maize (Aguirre et al. 2023), changes in stomatal aperture might have affected maize
305 perception and responses to neighbor's volatiles. In other words, FR-treated plants might take up more
306 volatiles than high R:FR-treated plants, resulting in a stronger priming stimulus. Alternatively, FR
307 positive effects on photosynthesis and stomatal opening might have increased the availability of
308 precursors for volatile biosynthesis (Arimura et al. 2008) and/or the release rate of these volatiles.
309 Further studies are needed to determine the mechanisms involved in FR-mediated modulation of plant
310 perception and responses to HIPVs.

311 Next, we showed that maize plants emit more HIPVs after pre-exposure to constitutive or
312 herbivory-induced volatiles from low R:FR-treated plants. A possible explanation for this phenomenon
313 is that a higher emission of volatiles by low R:FR-treated plants might trigger a stronger priming
314 response in the receiver. For instance, exposure to increasing concentrations of GLVs has been shown
315 to parallel increases in plasma membrane potential depolarization and cytosolic calcium concentrations
316 in plant leaves (Zebelo et al. 2012; Aratani et al. 2023). These cellular responses are associated with
317 the activation of JA defense-related genes (Aratani et al. 2023) and might be part of the priming stimulus
318 in the receiver plant. Our analysis of GLV emissions in intact plants and plants subjected to simulated
319 herbivory showed slightly higher levels of hexenal in low R:FR-treated plants compared to the high
320 R:FR treatment, but no differences in hexenyl acetate and hexenol emissions. Thus, it is unlikely that
321 these differences are responsible for the increase in HIPVs observed in receiver plants.

322 Additionally, our previous study revealed that an enriched FR light environment enhances the
323 emission of monoterpenes and the homoterpene DMMT in herbivory-induced B73 maize plants
324 (Escobar-Bravo et al. 2024). Yet, in the same experiments, we did not observe significant differences
325 in constitutive volatile emissions in unwounded FR-treated plants compared to high R:FR light
326 conditions (Escobar-Bravo et al. 2024). As both unwounded and herbivory-induced low R:FR treated
327 plants enhanced the volatile emissions in receiver plants, they might emit a common volatile signal that
328 was not detected in our PTR-TOF-MS system. This would be the case of volatile compounds that cannot
329 be ionized by the primary ion (H_3O^+) used in our system. A candidate signal could be the volatile
330 hormone ethylene. Ethylene emission is induced by low R:FR light conditions in several plant species,

including *Arabidopsis thaliana* and *Nicotiana tabacum*, playing an important role in the development of shade avoidance traits (Pierik et al. 2003, 2004; Kegge et al. 2013). Furthermore, ethylene exposure has been shown to increase the emission of HIPVs in maize plants when these were challenged with simulated herbivory (Schmelz et al. 2003) or (*Z*)-3-HOL exposure (Ruther and Kleier 2005). Further analyses determining ethylene emissions in low R:FR-treated maize plants together with the artificial manipulation ethylene signaling could shed light on the role of this volatile hormone in the stronger volatile-mediated priming response under enriched FR light conditions.

In summary, our findings suggest an enhanced transmission of volatile signals within maize canopies via FR-mediated effects on the emitter's volatile emissions and the perception of these volatiles by the receiver. Additional experiments are needed to determine (1) whether this translates into a larger spatial distribution of warning volatile signals in maize fields, (2) if this information transfer is species-specific, and (3) what are the benefits and tradeoffs of such a phenomenon. Under field conditions, for instance, the propagation of volatile signals involving several plants located at different distances from the emitter might be affected by the diffusion properties and breakdown of volatile compounds after release (Niinemets et al. 2014; Schuman 2023), as well as by their absorption and reabsorption by the plant/s closer to the volatile source (Himanen et al. 2010; Sugimoto et al. 2014, 2023). In addition, maize plants might be exposed to other plant-competition cues such as limited water and nutrient resources, and root-derived exudates from neighboring plants (Pierik et al. 2013; Wang et al. 2021) that might affect volatile emissions and perception. For instance, drought can modulate herbivory-induced (Catola et al. 2018; Lin et al. 2022; Vázquez-González et al. 2022) and constitutive volatile emissions (Jin et al. 2021), the latter affecting the drought tolerance in neighboring plants (Jin et al. 2021). Finally, we hypothesize that an enhanced transmission of volatile priming signals in maize fields might increase HIPVs emission at the plant community level which can boost the attraction of the natural enemies of herbivores (Aartsma et al. 2017). This might be especially relevant in dense monocultures, where the limited air movement within a dense canopy can reduce the dispersion of plant volatile compounds, potentially leading to volatile entrapment. Thus, our study not only provides a better understanding of the environmental modulation of the transmission of volatile priming signals in an agronomic relevant species, but also sets the basis to further determine their ecological functions.

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COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose

AUTHORS CONTRIBUTION

ME conceived the study. ME, RE-B, BCJS designed experiments. RE-B and BCJS conducted the experiments. RE-B and BCJS performed the PTR-TOF-MS measurements and analyses. RE-B analyzed the data. RE-B, BCJS, and ME interpreted the data. RE-B 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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499 **SUPPLEMENTARY MATERIAL**

500 **Figure S1.** Light spectral composition determined in far-red supplementation experiments.

501 **Figure S2.** Detailed schematic overview of the experimental set-up in Fig. 1

502 **Figure S3.** Detailed schematic overview of the experimental set-up in Fig. 2.

503 **Figure S4.** Detailed schematic overview of the experimental set-up in Fig. 3 and Fig. 4.