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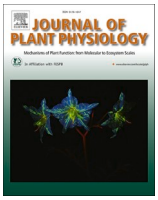

Cowpea volatiles induced by beet armyworm or fall armyworm differentially prime maize plants

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ABSTRACT

Exposure to herbivore-induced plant volatiles (HIPVs) is known to enhance the defense responses in plants. This so-called priming effect has only been marginally studied in intercropping systems. We tested whether HIPVs from cowpea, which often serves as an intercrop alongside maize, can prime herbivore-induced volatile emissions in maize. Conventional volatile collection assays and real-time mass spectrometry revealed that maize plants that were exposed to HIPVs from cowpea infested with *Spodoptera exigua* caterpillars emitted more than control plants when they themselves were subsequently damaged by the same pest. The enhanced emission was only evident on the first day after infestation. Maize plants that were exposed to HIPVs from cowpea infested by *S. frugiperda* larvae showed no priming effect and released considerably less upon *S. frugiperda* infestation than upon *S. exigua* infestation. The latter may be explained by the fact that *S. frugiperda* is particularly well adapted to feed on maize and is known to suppress maize HIPV emissions. Our results imply that HIPVs from cowpea, depending on the inducing insect herbivore, may strongly prime maize plants. This deserves further investigation, also in other intercropping systems, as it can have important consequences for tritrophic interactions and crop protection.

1. Introduction

Plants emit an array of herbivore-induced plant volatiles (HIPVs) upon insect attack. These inducible volatiles can have an indirect defense function by attracting the natural enemies of the herbivores (Dicke and Baldwin, 2010; Heil, 2014; Turlings and Erb, 2018). Some of these volatiles are also known to be perceived by the intact parts of the infested plants and by neighboring plants, which then exhibit faster and/or stronger defense responses upon subsequent herbivory (Frost et al., 2008; Karban et al., 2003; Kessler et al., 2006). This readying of a defense response is referred to as *priming* (Mauch-Mani et al., 2017). Upon priming, plant defenses are potentiated to provide faster and stronger defense responses for a subsequent insect attack, which is a cost-effective way to resist unpredictable attackers (van Hulten et al., 2006). Much of the evidence for VOC-mediated priming comes from studies on maize plants (*Zea mays*) (Engelberth et al., 2004; Erb et al., 2015; Ton et al., 2007). There are several classes of HIPVs acting as priming agents such as green leaf volatiles (GLVs), terpenoids, alcohols, esters, aldehydes, and several aromatic volatiles including indole (Cofer et al., 2018; Erb et al., 2015; Ye et al., 2021). For example, exposure of maize plants to the GLV (*Z*)-3-hexenol leads to upregulation of several genes in maize particularly involved in defense signaling including *ZmWRKY12* and *ZmMAPK6* (Engelberth et al., 2013). Exposing intact tea leaves to two terpenoids, α-farnesene and β-ocimene, has been shown to elicit metabolic changes associated with defensive functions (Zeng et al., 2017). The aromatic volatile compound indole has been shown to be essential for priming in maize (Erb et al., 2015). Furthermore, combined exposure to (Z)-3-hexenyl acetate and indole leads to a synergistic induction of benzoxazinoids that promote direct defense against numerous insect pests in maize (Hu et al., 2019). It is also known that HIPVs released upon infestation by caterpillars prime not only for direct, but also indirect defenses in maize. This involves the elicitation of a subset of defense-related genes, as well as a rapid and enhanced emission of HIPVs upon caterpillar attack (Ton et al., 2007). This priming of defense-related gene expression in maize leads to reduced caterpillar feeding and development and an earlier production of HIPVs upon caterpillar attack, increasing the attractiveness of the plants to parasitoids (Ton et al., 2007). It is generally agreed that the significance

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of plant-herbivore interactions mediated by volatile compounds is determined by the level of herbivore pressure and its consequential impact on plant fitness within the ecosystem (Karban, 2007).

In agricultural ecosystems, priming is known to occur in intercropping and monocropping systems (Shrivastava et al., 2010). Intercropping involves the cultivation of two or more crop species together in the same space for a certain period of time, often benefitting both crop species (Shrivastava et al., 2010), including the enhancement of yields per unit area (Latati et al., 2014). Intercropping has also been recommended as a means to curb pest outbreaks (Altieri et al., 1978; Dempster and Coaker, 1972). For instance, VOCs emitted from potato plants (*Solanum tuberosum*) in monocropping systems attract Colorado potato beetles (*Leptinotarsa decemlineata*), but VOC mixtures released when potato and tomato (*Lycopersicon esculentum*) are planted together are either repellent or not attractive to Colorado potato beetles (Thiery and Visser, 1986, 1987). A study by Skovgård and Päts (1997) revealed that combined cropping of maize and cowpea (*Vigna unguiculata*), which serves as a common intercrop in maize agroecosystems (Latati et al., 2014), can lead to substantial reduction in stem borer attack in maize.

The above studies analyzed the quantitative changes in unit crop yield and reductions in damage inflicted by pests in intercropping systems as compared to monocropping systems. However, to what extent intercrops can prime crops for enhanced VOC signaling in response to subsequent herbivory remains unknown. The phenomenon of priming may be highly relevant in the context of indirect defenses of maize against pests. As cowpea is generally intercropped with maize, it is pertinent to determine whether HIPVs released from cowpea plants can prime maize plants. Here, we studied this for two lepidopteran pests of maize, beet armyworm and fall armyworm, by determining whether exposure to different types of HIPVs modifies inducible volatile profiles in the receiving maize plants.

The beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae), is a generalist pest that is harmful to numerous crops, including maize (Blanco et al., 2014; De Lange et al., 2020). The fall armyworm, *S. frugiperda* (Lepidoptera: Noctuidae) is one of the most destructive pests of maize (Glauser et al., 2011; Sparks, 1979). It has recently invaded Africa and Asia, where it is causing significant losses in maize yield (Day et al., 2017) and has led to a tremendous increase in pesticide use (Rani et al., 2021). *S. frugiperda* can successfully overcome direct defenses in maize and is characterized as a maize specialist (Glauser et al., 2011; Wouters et al., 2014). In the Americas, *S. exigua* and *S. frugiperda* co-occur in maize agroecosystems (Blanco et al., 2014). *Spodoptera exigua* and *S. frugiperda* also readily feed on cowpea plants (Capinera, 2020; Costa et al., 2020).

Both *S. exigua* and *S. frugiperda* elicit HIPVs emissions from maize plants, but as a specialist pest on maize, *S. frugiperda* appears to partially suppress these emissions and induces considerably less compared to *S. exigua* (De Lange et al., 2020). However, there is no information on whether these differences have any effect on priming and to what extent exposure to HIPVs can overcome the distinctive volatile-suppression ability of *S. frugiperda* in maize. Therefore, the present study aimed to first examine if airborne signals emitted by cowpea and maize upon *S. exigua* and *S. frugiperda* infestation differentially prime maize plants in terms of timing and magnitude of volatile emissions due to subsequent herbivory. Secondly, we determined if priming of maize plants with different blends of HIPVs from cowpea and maize has any distinguishable impact on diurnal periodicity of VOC release throughout the period of a prolonged *S. exigua* or *S. frugiperda* attack. Finally, we explored the possibility that priming maize plants with HIPVs from cowpea or maize volatiles can overcome the volatile-suppression potential of *S. frugiperda*. For this, we studied the emission dynamics of HIPVs released from maize plants that were primed with airborne signals of cowpea and maize, through early and late phases of infestation by *S. exigua* and *S. frugiperda*.

2. Material and methods

2.1. Plant material and insects

Maize (*Z. mays* ssp. *mays*, variety DFI45321) seeds and cowpea (*V. unguiculata*, variety California blackeye) seeds were individually sown in plastic pots (10 cm high, 4 cm diameter) filled with commercial potting soil (Ricoter Erdaufbereitung AG, Aarberg, Switzerland). The plants were kept in a greenhouse starting from germination till they were used for the experiments. The standard environmental conditions in the greenhouse were: temperature of 25 ± 2 °C, light intensity of 300–400 μmol m^{-2} s^{-1} for 14 h photoperiod, ambient CO_2 concentration of 350–400 μmol mol^{-1}, and relative humidity of 60 ± 5 % throughout the experimental period. Seedlings were regularly watered to soil field capacity till the end of experiments. In all experiments, we used 9–13 days old maize seedings and 14–17 days old cowpea seedlings. At the beginning of the experiments, maize plants had 5 - 6 fully developed leaves and cowpea plants had 4–6 fully developed leaves.

The eggs of *S. exigua* and *S. frugiperda* were obtained from an in-house colony. Both species were reared in transparent plastic boxes on a wheat germ-based artificial diet (Frontier Scientific Services, Newark, USA) under laboratory conditions (25 ± 2 °C, 60% relative humidity, 16:8 h L/D) (Arce et al., 2021; Fallet et al., 2022).

2.2. Experimental design, priming, and volatile trapping

The maize and cowpea plants were enclosed in custom-made 3-port cylindrical glass chambers (2.5 L) for priming and volatile collection (Ton et al., 2007). Pure and humidified-ambient air was supplied to the glass chamber through the inlet port of the glass chamber at a rate of 1.1 l min^{-1}. The HIPVs were collected via the outlet port of the glass chamber for GC-MS or sampled with the probe of the PTR-TOF-MS (see below). To induce volatile emissions, emitter plants were infested with larvae of *S. exigua* or *S. frugiperda*, which were placed in the center of a plant. Cowpea plants that served as emitters were infested with seven third-instar larvae of *S. exigua* or ten third-instar larvae of *S. frugiperda* for 60 h before HIPVs were used to prime maize plants for 24 h. In this case, *S. exigua* consumed 7.3 ± 1.9 cm^2 (Mean ± SE) and *S. frugiperda* consumed 6.2 ± 1.6 cm^2 (Mean ± SE) of cowpea leaf. Maize plants that served as emitters were infested with seven third-instar larvae of *S. exigua* or four third-instar larvae of *S. frugiperda* for 36 h and then used to prime another batch of maize plants for 24 h. This resulted in about 9.2 ± 2.6 cm^2 (Mean ± SE) and 8.3 ± 1.3 cm^2 (Mean ± SE) of leaf consumption respectively. In the case of receiver plants, maize plants were fed upon by six third-instar larvae of *S. exigua* and three third-instar larvae of *S. frugiperda*. The numbers of caterpillars used for the infestations were based on the amount of damage that they inflicted in preliminary feeding assays. To obtain information on possible quantitative differences in the amounts released by these emitter plants, we collected their volatiles once the exposure treatments were completed and then quantified them using GC-MS. The duration of larval feeding on maize or cowpea plants prior to the exposure step was to get maximum HIPV emissions for priming, again based on preliminary assays.

There were five sets of priming experiments conducted in this study. In set I, the control maize plants were first exposed for 24 h to clean air and then fed upon by *S. exigua* or *S. frugiperda* larvae for 96 h (Fig. 1A and C). In set II, the maize plants were first exposed for 24 h to HIPVs released from cowpea or maize plants infested with *S. exigua* larvae and subsequently the exposed plants were infested with *S. exigua* larvae for 96 h (Fig. 1A). In set III, the maize plants were exposed for 24 h to HIPVs released from cowpea or maize plants that were infested with *S. frugiperda* larvae, and then the exposed plants were infested with *S. frugiperda* larvae for 96 h (Fig. 1C). In set IV, the maize plants were exposed for 24 h to HIPVs emitted from cowpea or maize plants infested with *S. frugiperda* larvae, and *S. exigua* larvae were then allowed to feed on the exposed plants for 72 h (Fig. 1B). In set V, the maize plants were

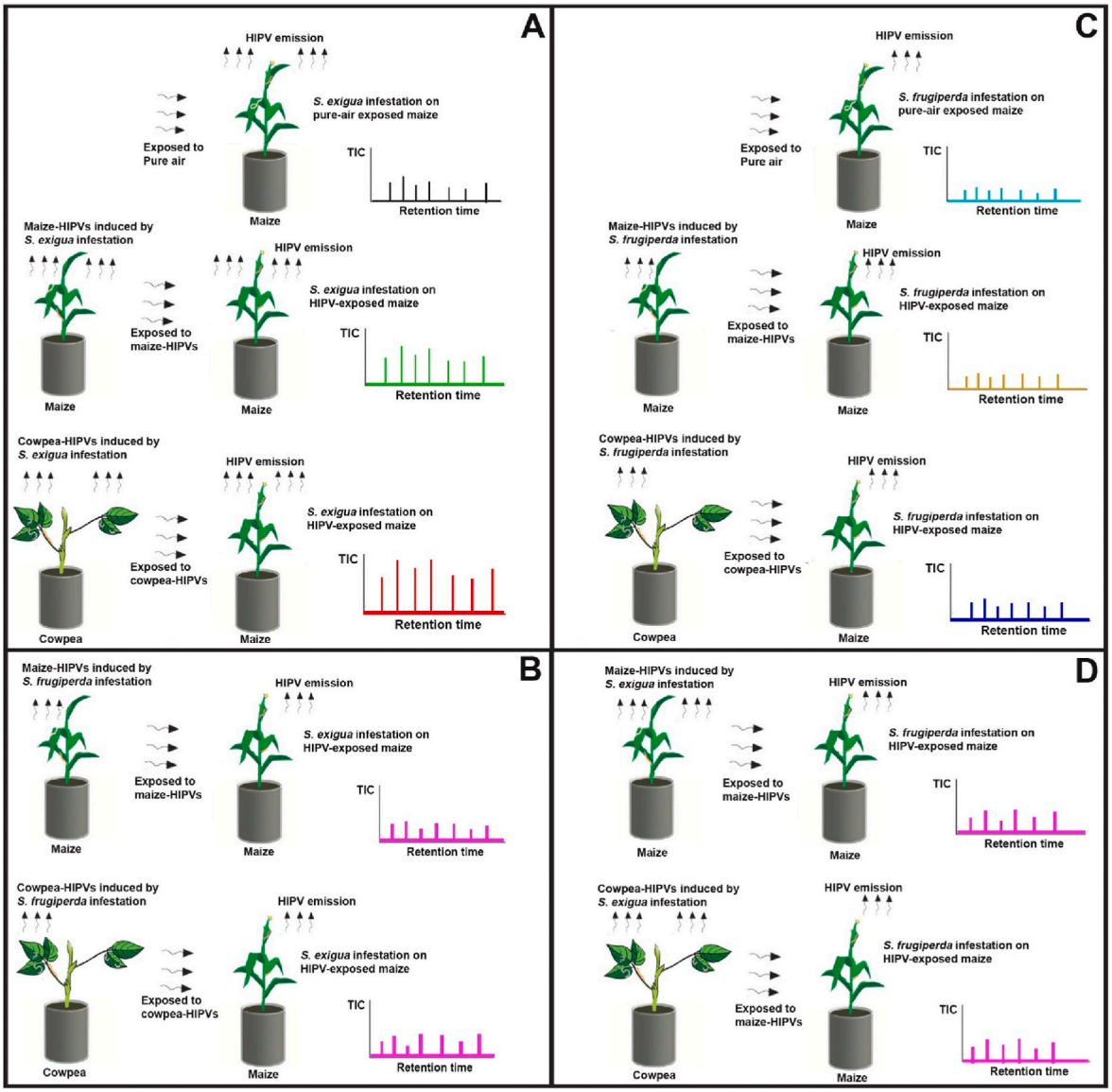

Fig. 1. Illustration of differential exposure of herbivore induced plant volatiles (HIPVs) to maize plants and the subsequent infestation of *S. exigua* and *S. frugiperda* larvae on differentially exposed-maize plants. The control maize plants were first exposed for 24 h to clean air and then fed upon by *S. exigua* or *S. frugiperda* larvae for 96 h (Fig. 1A and C). The maize plants were first exposed for 24 h to HIPVs released from cowpea or maize plants infested with *S. exigua* larvae and the exposed-plants were subsequently infested with *S. exigua* larvae for 96 h (Fig. 1A). The maize plants were exposed for 24 h to HIPVs released from cowpea or maize plants that were initially infested with *S. frugiperda* larvae, and then the exposed-plants were infested with *S. frugiperda* larvae for 96 h (Fig. 1C). The maize plants were exposed for 24 h to HIPVs emitted from cowpea or maize plants initially infested with *S. frugiperda* larvae, and then *S. exigua* larvae were allowed to feed on the exposed-plants for 72 h (Fig. 1B). The maize plants were first exposed for 24 h to HIPVs emitted from cowpea or maize plants with *S. exigua* infestation, and then *S. frugiperda* larvae were placed on the exposed-plants for 72 h (Fig. 1D). The colour codes of illustrative chromatograms in Fig. 1A and C are in accordance with the colour codes of PTR-MS data in Fig. 5. There were six replicates for each treatment in this study. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

exposed for 24 h to HIPVs emitted from cowpea or maize plants with *S. exigua* infestation, and then *S. frugiperda* larvae were placed on the exposed plants for 72 h (Fig. 1D). The larvae remained on the emitter and receiver plants throughout the experimental period. The number of larvae were chosen on the basis of preliminary experiments (data not shown) to balance the differential leaf consumption by both species. In all cases, before using them to infest plants for the experiments, the larvae were first fed with the same variety of maize or cowpea plants for 24 h.

For the sets I - III of the study, the volatile sampling for GC-MS analysis of the exposed plants was carried out at 24, 48, 72, and 96 h of infestation by *S. exigua* or *S. frugiperda* larvae on differentially primed maize plants, and for the sets IV and V of the study, the volatile sampling was carried out at 24, 48, and 72 h of larval infestation. In all cases, volatiles were collected for 3h (see below). All connections to and from glass chamber were made of Teflon® and copper tubing in the volatile collection set-up (Analytical Research System, Gainesville, USA). The standard environmental conditions in the plant-enclosed glass chambers during the study were: temperature of 25 ± 2 °C, PPFD of 400–500 µmol m^{-2} s^{-1} for 14 h photoperiod, ambient CO_2 concentration of 390–400 µmol mol^{-1}, and relative humidity of 60 ± 5 %.

2.3. Sample preparation for gas chromatography - mass spectrometry (GC-MS) analysis

Adsorbent filters containing the porous adsorbent material Porapak

Q (25 mg; 80–100 mesh; Ohio Valley Specialty Company, OH, USA) were used to trap volatiles for GC-MS analysis. Prior to use, the Porapak Q filters were rinsed with 5 × 200 µl of DCM (dichloromethane ≥99.9 %, Honeywell, Switzerland). In all cases, the HIPVs in the air sample were drawn through each Porapak Q filter at a rate of 0.4 l/min for 3 h through the outlet ports of the glass chambers, using a suction pump (HuberLab, Aesch, Switzerland).

After each sampling, volatiles adsorbed on the Porapak Q filters were extracted using 150 µl DCM (Honeywell, Switzerland). An amount of 200 ng each of n-octane and n-nonyl acetate (Sigma-Aldrich, Buchs, Switzerland) was added in 10 µl of solution (i.e., 20 ng/µl) to each filter extract as internal standards to quantify HIPVs. The samples were stored at −80 °C in 250 µl conical bottom inserts in 2 ml screw vials with 9 mm screw caps w/PTFE liner (Interchim, Montluçon, France) prior to analysis in GC-MS.

The volatile samples were analyzed in a gas chromatograph (Agilent 7890B) coupled to a mass spectrometer detector (Agilent 5977B), equipped with an HP-5ms GC column. Upon injection of 1.5 µl of sample, the GC oven temperature was kept at 40 °C for 3.5 min, and then increased up to 100 °C at the rate of 8 °C/min and eventually to 230 °C at the rate of 5 °C/min. This was followed by a post-run of 5 min at 250 °C prior to reaching initial conditions in GC. Helium at a constant flow of 0.9 ml/min was used as carrier gas. Mass spectrometry (MS) ionization was achieved by electron impact at an emission current of 30 µA in the ion trap held at 200 °C. MS data were collected in full scan mode with a mass range of m/z 40–300. The volatiles were identified by comparing their mass spectra with those of NIST 05 library and pure standards.

2.4. Real-time monitoring of the kinetics of HIPVs by proton-transfer reaction-time of flight mass spectrometry (PTR-TOF-MS)

A high-resolution PTR-TOF-MS (Model: Vocus S; Tofwerk AG, Switzerland) was used to detect possible differences in diurnal cycle of emission responses of HIPVs upon differential priming. HIPVs in the air can be detected in real time by PTR-TOF-MS upon proton reactions occurring between hydronium ions (H_3O^+) produced within the discharge ion source and the air sample (Li et al., 2020).

The air exiting the outlet of the glass chamber containing the experimental plant was continuously analyzed in the PTR-TOF-MS for 96 h, starting at the onset of caterpillar infestation on differentially primed maize plants. The data recording by the PTR-TOF-MS was started as soon as a steady airflow was achieved, ca. 10 min after the enclosure of the maize plant into the glass chamber. The PTR-TOF-MS analyses were carried out with a single plant for each treatment. In all cases, pure and humidified-ambient air was supplied to the glass chamber at a rate of 1.1 l min^{-1} and the air from the glass chamber was drawn into the PTR-TOF-MS through a suction tube at a rate of 0.1 l min^{-1}. The sensitivity of the PTR-TOF-MS instrument was 10000 cps/ppb, and the mass resolving power 7000 m/Δm. The details about the instrument, operation, and system calibration can be found in Li et al. (2020) and Hilfiker et al. (2019). In brief, the ion molecular reactor (IMR) conditions during operation were: pressure of 1.8 mbar, and temperature of 100 °C, IMR front voltage of 500 V, and IMR back voltage 38 V. The data were recorded at a time resolution of 15 s. Prior to each measurement, the PTR-TOF-MS was calibrated using a tank with a blend of calibration VOC standards (Apel-Riemer Environmental Inc., USA), consisting of acetaldehyde, methanol, acetonitrile, acetone, acrylonitrile, isoprene, methyl-ethyl ketone, benzene, toluene, *m*-xylene, α-pinene, 1,2,4-trimethylbenzene, and β-caryophyllene. The compounds in the calibration gas mixture covered the mass-to-charge (*m/z*) values ranging from 33 to 205. The raw PTR-TOF-MS data were acquired and post-processed with Tofware 3.2.1 (Tofwerk AG, Switzerland) including peak-fitting of targeted ions to avoid overlapping peaks.

2.5. Data analysis

The impact of larval infestations on temporal emission rate of HIPVs released from differentially primed maize plants was statistically analyzed with generalized linear models (GLMs) in RStudio (v.1.4.1106). GLMs fitted with gamma distribution were used to estimate individual and interactive effect of priming by cowpea volatiles (Cowpea), maize volatiles (Maize), and larval infestation time (24, 48, 72, and 96 h after the onset of caterpillar infestation) on HIPV emissions from maize plants in response to *S. exigua* and *S. frugiperda* infestations. The selection of GLM was done by comparing Akaike Information Criterion values. Distribution of datasets were identified by checking QQplots, histograms, and also using the functions shapiro.test and qqnorm (residuals ()). In all cases, the emissions of HIPVs from maize plants, which were exposed to pure and humidified-ambient air for 24 h and then treated with *S. exigua* and *S. frugiperda* were considered as controls. This part of the study was replicated six times. The graphs were plotted using SigmaPlot (v. 12.5; Systat Software Inc, San Jose, USA).

The PTR-TOF-MS measurement (section 2.4) was replicated once, and the graphs were plotted in RStudio (v.1.4.1106), using ggplot2 package (Wickham, 2016).

3. Results

3.1. Emission of HIPVs from differentially primed maize plants in response to S. exigua and S. frugiperda infestation

In general, the maize plants, which were exposed to HIPVs of *S. exigua*-infested cowpea plants and then infested with *S. exigua* emitted substantially higher amounts of total homo-, mono- and sesquiterpenes, as well as indole, compared to control plants (Table 1, Figs. 2 and 3, and S. Table). More specifically, the enhanced emissions were only evident the day after larval infestation on differentially primed maize plants (Fig. 2A–C). This was not the case for *S. frugiperda* infestation, where maize plants primed with HIPVs of *S. frugiperda*-infested cowpea plants and then treated with *S. frugiperda* did not show any dramatic enhancement of different classes of total VOC emission, except for total sesquiterpene emissions compared with controls (Table 1, Figs. 2 and 3, and S. Table). Counter-intuitively, increases in the total GLV emission rates were not statistically significant upon both pest infestations ($P > 0.05$) (Table 1 and Fig. 2).

GLM analysis further indicated that the exposure of maize plants to HIPVs of *S. exigua*-damaged cowpea plants and subsequent infestation by *S. exigua* led to considerably higher emission rates of several volatile compounds than those from maize plants exposed to HIPVs of *S. exigua*-damaged maize plants, specifically (Z)-3-hexen-1-ol, (*Z*)-3-hexen-1-ol acetate, (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), (*E,E*)-4,8,12-trimethyltrideca-1,3,7,11- tetraene (TMTT), (*S*)-linalool, β-myrcene, (*E*)-β-ocimene, (*E*)-α-bergamotene, (*E*)-β-caryophyllene, and indole (Tables S1 and S5). The same was true, but to a relatively lesser extent, for maize plants primed with HIPVs from *S. frugiperda*-infested cowpea plants that were subsequently subjected to *S. frugiperda* infestations. This was specifically the case for (*Z*)-3-hexenal, β-myrcene, (*E*)-α-bergamotene, (*E*)-β-caryophyllene, and (*E*)-β-farnesene (Tables S2 and S5). In fact, the number of volatile compounds that were substantially released were emitted by maize plants primed with cowpea-volatiles and were higher than those emitted by maize plants primed with maize-volatiles, and subsequently infested with either pest (Table S1, S2 and S5).

To further elucidate whether quantitative differences in HIPVs had an effect on the degree of priming in maize plants, we primed maize plants with HIPVs emitted from cowpea or maize plants damaged by *S. exigua* larvae, and subsequently subjected the primed-maize plants to *S. frugiperda* infestation and vice versa. Interestingly, the maize plants exposed to HIPVs emitted by *S. exigua*-infested cowpea and then fed upon by *S. frugiperda* released considerably higher quantities of

Table 1
Summary statistics of generalized linear models (GLMs) for different classes of herbivore induced plants volatiles, and indole. GLMs fitted with gamma distribution were used to estimate individual and interactive effect of exposure of cowpea volatiles (Cowpea) and maize volatiles (Maize) to maize plants, and larval infestation time (Time; 24, 48, 72, and 96 h after the onset of caterpillar infestation) on HIPV emissions from differentially-exposed maize plants upon *S. exigua* and *S. frugiperda* infestations. The details of differential exposure, larval treatments, as illustrated in Fig. 1A and C. The data presentation as in Tables S1 and S2. *P*-values show the statistical significance of treatments, demonstrated as: -$P < 0.01$ (**), and -$P < 0.001$(***).

	S. exigua infestation				*S. frugiperda* infestation			
Treatment	Estimate	Std. error	t-value	P-value	Estimate	Std. error	t-value	P-value
Total GLV emission rate								
Cowpea	0.694	0.664	1.044	0.300	24.263	15.353	1.580	0.118
Maize	−0.143	0.664	−0.216	0.830	46.459	25.465	1.824	0.072
Time	−0.007	0.007	−1.072	0.287	**1.092**	**0.367**	**2.973**	**0.004****
Cowpea: Time	−0.000	0.010	−0.064	0.949	−0.776	0.428	−1.815	0.074
Maize: Time	0.005	0.010	0.534	0.595	−0.804	0.543	−1.482	0.143
Total homoterpene emission rate								
Cowpea	**97.149**	**29.431**	**3.301**	**0.001****	0.676	0.551	1.228	0.224
Maize	21.272	16.157	1.317	0.192	0.488	0.551	0.887	0.378
Time	−0.163	0.114	−1.425	0.158	0.002	0.005	0.461	0.646
Cowpea: Time	**−0.986**	**0.329**	**−2.996**	**0.003****	−0.005	0.008	−0.709	0.481
Maize: Time	−0.198	0.201	−0.984	0.328	0.002	0.008	0.260	0.796
Total monoterpene emission rate								
Cowpea	**272.789**	**76.952**	**3.545**	**< 0.000*****	14.401	11.900	1.210	0.231
Maize	63.131	41.592	1.518	0.133	20.877	15.526	1.345	0.183
Time	−0.121	0.224	−0.543	0.589	0.159	0.113	1.408	0.164
Cowpea: Time	**−2.750**	**0.855**	**−3.216**	**0.002****	−0.115	0.196	−0.589	0.558
Maize: Time	−0.243	0.583	−0.417	0.677	−0.015	0.260	−0.059	0.953
Total sesquiterpene emission rate								
Cowpea	**976.820**	**331.006**	**2.951**	**0.004****	**92.854**	**30.251**	**3.069**	**0.003****
Maize	179.367	187.707	0.956	0.342	36.326	30.396	1.195	0.236
Time	1.539	1.577	0.976	0.332	**1.355**	**0.388**	**3.486**	**< 0.000*****
Cowpea: Time	**−11.232**	**3.885**	**−2.891**	**0.005****	**−1.722**	**0.533**	**−3.230**	**0.001****
Maize: Time	−0.727	3.087	−0.236	0.814	−0.180	0.681	−0.265	0.791
Indole								
Cowpea	**395.526**	**146.756**	**2.695**	**0.008****	−8.852	21.621	−0.409	0.683
Maize	10.424	62.762	0.166	0.868	−23.837	20.151	−1.183	0.241
Time	−0.699	0.422	−1.655	0.102	−0.326	0.168	−1.939	0.056
Cowpea: Time	**−4.160**	**1.556**	**−2.673**	**0.009****	0.313	0.301	1.040	0.302
Maize: Time	0.367	0.845	0.435	0.665	0.605	0.312	1.938	0.056

volatiles, compared to maize plants exposed to HIPVs from *S. frugiperda*-infested cowpea and were then challenged by the same species (Tables 1, 2, S2 and S4, and Fig. 4). In contrast, the maize plants, which were exposed to HIPVs from *S. frugiperda*-infested cowpea or maize, and then challenged by *S. exigua* larvae emitted lower amounts of volatiles than maize plants exposed to HIPVs from *S. exigua*-damaged cowpea or maize and then subjected to the same larval species (Tables 1, 2, S1 and S3, and Fig. 4). Based on these observations, it is therefore conceivable that quantitative differences in HIPVs used to prime maize plants play a key role in determining the strength of priming (S. Tables 6 and 7). However, we cannot rule out any undetectable and unidentified volatile compounds that are likely released from cowpea and maize plants in response to *S. exigua* or *S. frugiperda* infestation that may also impact priming of maize plants.

3.2. Real-time monitoring of the kinetics of stress volatiles from differentially primed maize plants

Using PTR-TOF-MS analyses, we studied the emission dynamics of GLVs, total mono- and sesquiterpenes, homoterpenes, and the dominant aromatic volatile, indole emitted from differentially primed maize plants throughout the period of larval infestation (Fig. 5). GLV emissions from primed maize plants were precisely captured by the following parent ions of exact masses; (*Z*)-3-hexenal and (*E*)-2-hexenal [*m/z* 99.080, $(C_6H_{10}O)H^+$], (*Z*)-3-hexen-1-ol [*m/z* 101.096, $(C_6H_{12}O)H^+$], and (*Z*)-3-hexenyl acetate [*m/z* 143.106, $(C_8H_{14}O_2)H^+$]. The GLV blend was dominated by (*Z*)-3-hexenal and (*E*)-2-hexenal, followed by (*Z*)-3-hexenyl acetate, and (*Z*)-3-hexen-1-ol, which was in accordance with the emission pattern of GLVs detected by GC-MS analysis (Fig. 5A–C, and Tables S1 and S2). In all cases, the highest GLV emissions were detected from a maize plant exposed to HIPVs of a cowpea plant, followed by those emitted from a maize plant exposed to HIPVs of a maize plant, in both cases upon subsequently infested by *S. exigua* (Fig. 5A–C). Furthermore, *S. frugiperda* infestation considerably curbed GLV emissions compared to those of *S. exigua* throughout the period of herbivory. Interestingly, the emissions of (*Z*)-3-hexenal & (*E*)-2-hexenal and 3-hexen-1-ol were consistent, irrespective of a diurnal periodicity (Fig. 5A and C), but (*E*)-3-hexenayl acetate emission was diurnally regulated (Fig. 5B).

The emissions of homoterpenes, DMNT and TMTT, were represented by the protonated parent ions [*m/z* 151.148, $(C_{11}H_{18})H^+$] and [*m/z* 219.210, $(C_{16}H_{26})H^+$] respectively, which were diurnally regulated (Fig. 5D and E). PTR-TOF-MS also detected monoterpenoid emissions based on the parent ion of *m/z* 137.132 [$(C_{10}H_{16})H^+$] for β-myrcene and (*Z*)-β-ocimene, and *m/z* 155.143 [$(C_{10}H_{18}O)H^+$] for (*S*)-linalool. In the case of (*S*)-linalool, our preliminary analysis indicated that ca. 96% of protonated (*S*)-linalool [$(C_{10}H_{18}O)H^+$] was fragmented into [$(C_{10}H_{16})H^+$]. Therefore, the total monoterpenes emissions from differentially primed-maize plants were reported as the sum of [$(C_{10}H_{18}O)H^+$] and [$(C_{10}H_{16})H^+$] (Fig. 5F). In the case of sesquiterpenoids, (*E*)-nerolidol was detected by the parent ion *m/z* 223.205 [$(C_{15}H_{26}O)H^+$] and all other sesquiterpenes were detected by the parent ion of *m/z* 205.195 [$(C_{15}H_{24})H^+$]. Thus, the total sesquiterpenoid emissions were indicated as the sum of [$(C_{15}H_{26}O)H^+$] and [$(C_{15}H_{24})H^+$] (Fig. 5G). The highest terpenoid emissions were observed from a maize plant primed with HIPVs from a cowpea plant, followed by those released from a maize plant primed with HIPVs of a maize plant, both upon *S. exigua* infestation (Fig. 5D–G). The highest indole [*m/z* 118.065, $(C_8H_7N)H^+$] emission were observed from a maize plant primed with HIPVs from a *S. exigua* -infested cowpea plant, followed by a maize plant primed with HIPVs from a *S. exigua*-infested maize plant (Fig. 5H). In accordance with GC-MS analysis, PTR-TOF-MS analysis further showed that the

Table 2
Summary statistics of generalized linear models (GLMs) for total and different classes of herbivore induced plants volatiles, and indole. GLMs fitted with gamma distribution were used to estimate individual and interactive effect of exposure of cowpea volatiles (Cowpea) and maize volatiles (Maize) to maize plants, and larval infestation time (Time; 24, 48, and 72 after the onset of caterpillar infestation) on HIPV emissions from differentially-exposed maize plants upon *S. exigua* and *S. frugiperda* infestations. The details of differential exposure, and larval treatments as illustrated in Fig. 1B and D. The data presentation as in Tables S3 and S4. *P*-values show the statistical significance of treatments, demonstrated as: $P < 0.05$ (*), and -$P < 0.01$ (**).

	S. exigua infestation				*S. frugiperda* infestation			
Treatments	Estimate	Std. error	t-value	P-value	Estimate	Std. error	t-value	P-value
Total HIPV emission rate								
Cowpea	312.054	362.693	0.860	0.394	335.439	168.824	1.987	0.052
Maize	105.188	306.590	0.343	0.733	**407.217**	**187.801**	**2.168**	**0.035***
Time	6.687	4.723	1.416	0.163	**5.459**	**2.063**	**2.647**	**0.011***
Cowpea: Time	−2.916	7.862	−0.371	0.712	−5.303	3.699	−1.434	0.158
Maize: Time	−1.260	6.980	−0.180	0.858	−6.399	3.882	−1.648	0.105
Total GLV emission rate								
Cowpea	−0.910	0.575	−1.582	0.120	**60.110**	**24.512**	**2.452**	**0.017***
Maize	−0.281	0.575	−0.489	0.627	42.351	27.807	1.523	0.134
Time	0.002	0.007	0.296	0.768	**1.517**	**0.538**	**2.819**	**0.006****
Cowpea: Time	0.013	0.011	1.222	0.228	**−1.545**	**0.660**	**−2.342**	**0.023***
Maize: Time	−0.011	0.011	−1.060	0.294	−0.811	0.797	−1.018	0.313
Total homoterpene emission rate								
Cowpea	**55.731**	**25.823**	**2.158**	**0.035***	**17.761**	**8.544**	**2.079**	**0.043***
Maize	22.978	18.844	1.219	0.228	**24.106**	**9.583**	**2.515**	**0.015***
Time	0.186	0.206	0.904	0.370	**0.284**	**0.105**	**2.682**	**0.010***
Cowpea: Time	−0.729	0.448	−1.628	0.110	−0.281	0.185	−1.522	0.134
Maize: Time	−0.264	0.368	−0.719	0.475	**−0.399**	**0.190**	**−2.103**	**0.040***
Total monoterpene emission rate								
Cowpea	45.245	42.236	1.071	0.289	40.388	20.266	1.993	0.051
Maize	−2.785	28.053	−0.099	0.921	**65.705**	**24.371**	**2.696**	**0.009****
Time	0.357	0.366	0.977	0.334	**0.542**	**0.218**	**2.481**	**0.016***
Cowpea: Time	−0.037	0.874	−0.043	0.966	−0.568	0.423	−1.342	0.185
Maize: Time	0.475	0.666	0.714	0.479	**−1.043**	**0.440**	**−2.368**	**0.021***
Total sesquiterpene emission rate								
Cowpea	161.399	160.438	1.006	0.319	103.414	79.808	1.296	0.201
Maize	−39.152	124.789	−0.314	0.755	98.209	74.361	1.321	0.192
Time	**5.141**	**2.290**	**2.245**	**0.029***	**2.226**	**0.951**	**2.340**	**0.023***
Cowpea: Time	−1.546	3.961	−0.390	0.698	−1.086	1.922	−0.565	0.574
Maize: Time	2.318	3.778	0.613	0.542	−1.178	1.811	−0.650	0.518
Indole emission rate								
Cowpea	**151.977**	**73.003**	**2.082**	**0.042***	**80.884**	**40.086**	**2.018**	**0.049***
Maize	**184.600**	**83.990**	**2.198**	**0.032***	**142.111**	**53.324**	**2.665**	**0.010***
Time	0.726	0.691	1.051	0.298	**0.775**	**0.379**	**2.044**	**0.046***
Cowpea: Time	**−2.540**	**1.236**	**−2.055**	**0.045***	−1.257	0.757	−1.660	0.103
Maize: Time	**−2.895**	**1.388**	**−2.086**	**0.042***	**−2.316**	**0.856**	**−2.706**	**0.009****

enhanced VOC emissions were most obvious after 24 h of *S. exigua* and *S. frugiperda* infestations on HIPV-exposed maize plants (Figs. 2, 3 and 5).

3.3. Volatiles emitted from donor plants

To make a link between possible differential priming effects of the different HIPV blends that were tested, we also determined and quantified the volatiles that were emitted by the caterpillar-infested donor plants that were used for the exposure of the receiver plants. The emissions were measured just after the plants had been used in the exposure tests at 2.5 days for maize and at 3.5 days for cowpea after initial infestation. The results, as presented in Tables S6 and S7, respectively confirm that maize plants release more compounds and considerably larger quantities than cowpea, as previously shown (Hoballah et al., 2002). The tables also present the information on the average amount of damage inflicted to the leaves, which was similar for *S. exigua and S. frugiperda.* The blends released by maize and cowpea leaves had several volatile compounds in common, such as DMNT, TMTT, linalool, (*E*)-β-ocimene, (*E*)-β-farnesene, (*E*)-nerolidol and indole. Among these compounds, we can expect one or several to be highly relevant for the priming effects.

4. Discussion

Maize plants that were primed with HIPVs released from *S. exigua*-infested cowpea plants and then subjected to *S. exigua* infestation showed statistically significant increases in emission rates of several HIPVs (Table 1, S1, S3, and S5, and Figs. 2 and 3). In contrast, very few compounds showed clearly higher rates of emissions from differentially primed maize plants upon *S. frugiperda* infestation (Table 1, S2, S4 and S5, and Figs. 2 and 3). As was already reported by De Lange et al. (2020), the findings in our study further suggest that *S. frugiperda* feeding specifically suppresses the biosynthesis of HIPVs compared to *S. exigua* feeding even in primed-maize plants. In fact, De Lange et al. (2020) revealed that the suppressed induction of HIPVs is primarily caused by lower defense-elicitation potential of *S. frugiperda*-regurgitant and possibly differential leaf damage caused by larval feeding. Therefore, it is conceivable that certain compounds in the oral secretion of *S. frugiperda* act as suppressors (effectors) of herbivore-induced JA responses, curbing the biosynthesis and release of several HIPVs.

In general, the first type of volatiles to be released upon plant damage are GLVs. GLVs are characteristic for membrane-level damage of plant tissues resulting from abiotic and biotic stresses (Scala et al., 2013; Turlings et al., 1998). In our study, the sustained emission of abundant GLVs, particularly (*Z*)-3-hexenal, (*E*)-2-hexenal, and (*Z*)-3-hexenyl acetate confirms that there was continuous feeding activity on the maize plants by both herbivores throughout each day (Tables 1, 2, S1, S2, and Figs. 2 and 5). This phenomenon is particularly evident after 24 h of larval infestation. It can be expected that the enhanced GLV release from primed maize plants can further boost defense responses in maize against *S. exigua* and *S. frugiperda*. In support of

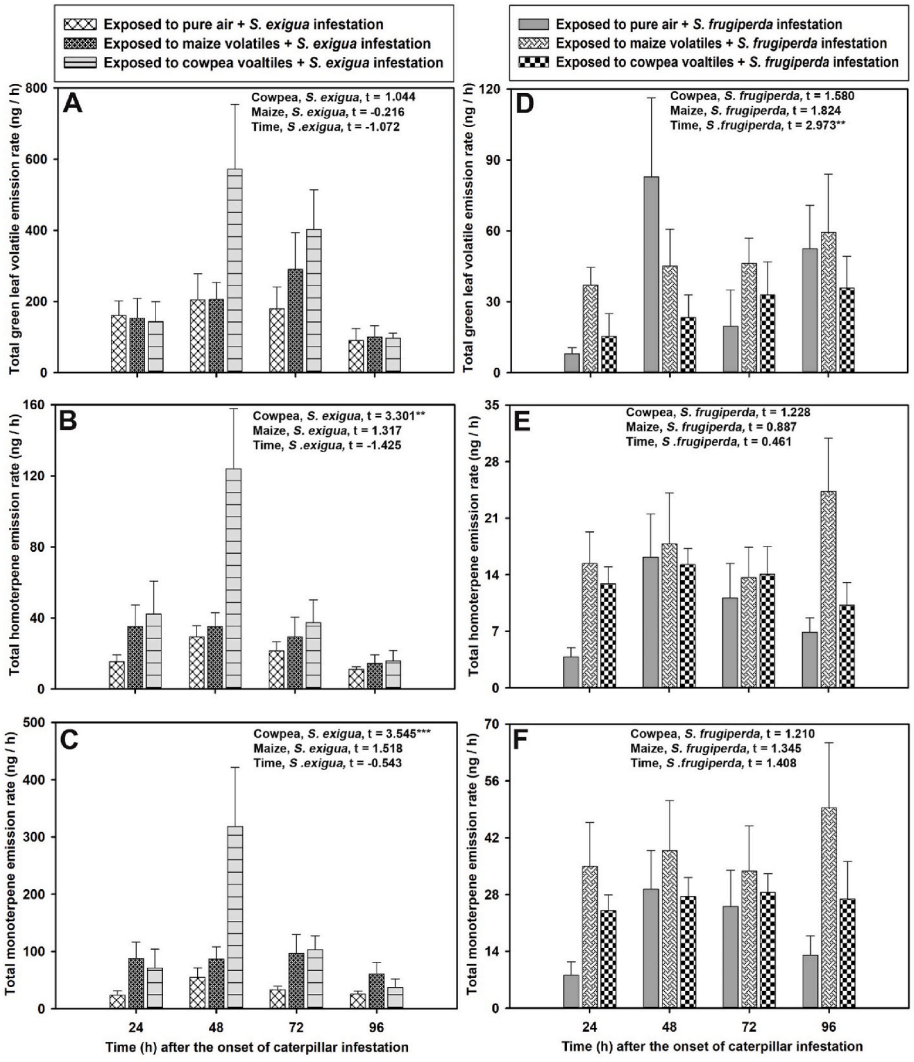

Fig. 2. Representative time-courses of emission rates (Mean + SE) of total green leaf volatiles (GLVs) (Fig. 2A and D), total homoterpenes (Fig. 2B and E), and total monoterpenes (Fig. 2C and F) released from differentially exposed-maize plants upon *S. exigua* (A, B, C) and *S. frugiperda* (D, E, F) infestations. There were six replicates for each treatment. Generalized linear models (GLM) fitted with gamma distribution were used to estimate individual and interactive effects of differential exposure and larval infestation time on HIPV emissions from differentially exposed-maize plants in response to *S. exigua* and *S. frugiperda* infestations. The data presentation as in Tables S1 and S2. The summary statistics of GLM as in Table 1.

this notion, Christensen et al. (2013) demonstrated that a blend of typical maize GLVs consisting of (*Z*)-3-hexenal, (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, and (*Z*)-3-hexenyl acetate significantly upregulated indirect defenses against *S. littoralis*. In particular, koinobiont parasitoids exploit theses volatile compounds for host location, as they signal the presence of hosts, already starting with very early instar infestation (Poirié et al., 2009; Vinson, 1976), various parasitoids of the *Spodoptera* caterpillars prefer early instar larvae as hosts (Hoballah et al., 2004). This highlights the potentially important role of GLVs as early defense signals. Moreover, herbivorous insects may chemically manipulate the isomerization of classical GLVs (Allmann and Baldwin, 2010; Allmann et al., 2013; Jones et al., 2019), underscoring the specificity and importance of GLVs in revealing the presence of insect herbivores, but also the potential of insects to manipulate their emissions and suppress defense signaling.

Plants also typically release various terpenoids in response to herbivory, which can be attractive to natural enemies of the herbivores (Sobhy et al., 2015; Tamiru et al., 2011). Overall, we found a substantial increase in the amounts of terpenoids released upon *S. exigua* feeding on maize plants primed with *S. exigua*-infested cowpea volatiles, considerably more than what was released from maize plants primed with *S. frugiperda*-infested cowpea volatiles and were then subjected to *S. frugiperda* infestation (Table 1, S1, S2, S5 and Figs. 2 and 3). Several terpenoids that were emitted in larger quantities from maize plants primed with HIPVs of cowpea have been linked to defensive roles against insect herbivores. For instance, some of the dominant herbivore-inducible terpenoids such as (*S*)-linalool, DMNT, TMTT, and (*E*)-β-caryophyllene have been reported to enhance indirect defenses against *Spodoptera* neonates attacking maize (Degenhardt and Gershenzon, 2000; Houshyani et al., 2013; Köllner et al., 2008). However, Rostás and Turlings (2008) reported that the most dominant herbivory-induced sesquiterpenes of maize such as (*E*)-β-farnesene and (*E*)-α-bergamotene (Schnee et al., 2006) or the abundant aromatic compound indole (D'Alessandro et al., 2006) are not essential for the innate attraction of common parasitoids of *Spodoptera* species, but these compounds do become attractive after the parasitoids learn to associate them with the presence of hosts (Fontana et al., 2011; Turlings and Erb, 2018). It appears that undetectable minor volatiles act alone or in combination with terpenoids for efficient attraction of natural enemies of herbivorous insects to host-infested maize plants (D'Alessandro et al., 2009).

Indole is a key airborne signal that primes for early defense signaling

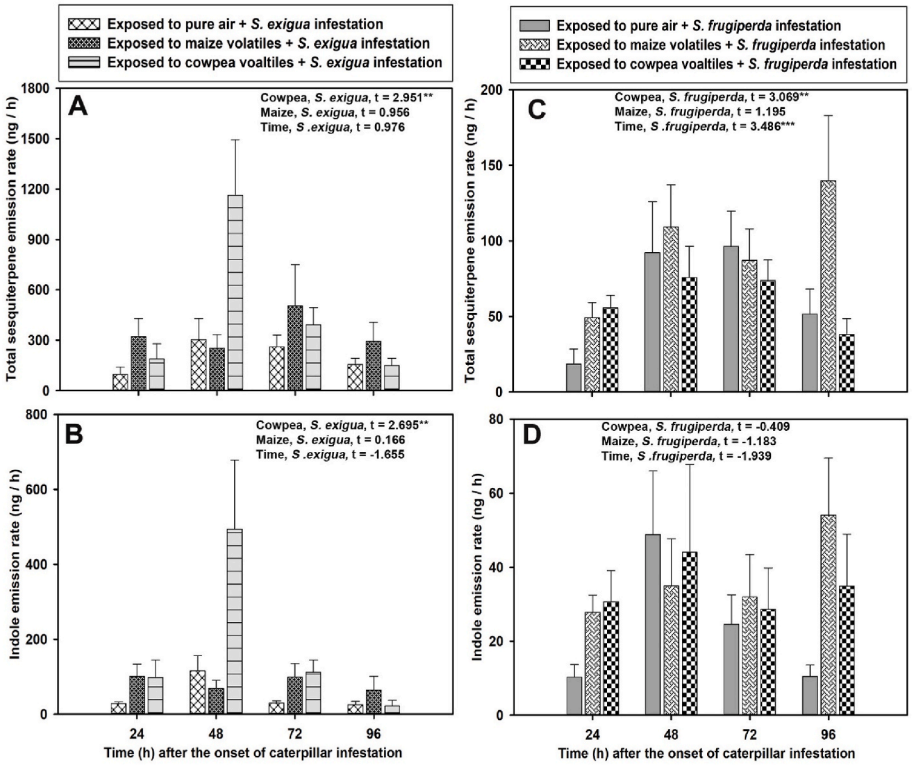

Fig. 3. Temporal variation in emission rates (Mean + SE) of total sesquiterpenes (Fig. 3A and C), and indole (Fig. 3B and D) released from differentially exposed-maize plants upon *S. exigua* (A, B) and *S. frugiperda* (C, D) infestations. There were six replicates for each treatment. Generalized linear models (GLM) fitted with gamma distribution were used to estimate individual and interactive effects of differential exposure and larval infestation time on HIPV emissions from differentially exposed-maize plants in response to *S. exigua* and *S. frugiperda* infestations. The data presentation as in S. Tables 1 and 2 The summary statistics of GLM as in Table 1.

in maize, especially for jasmonates and volatile terpenes (Erb et al., 2015; Hu et al., 2019). In particular, the combined exposure to indole and (*Z*)-3-hexenyl acetate triggers an enhanced production of benzoxazinoids which serve as the primary direct-defense compounds in maize (Hu et al., 2019). This implies that defense responses in maize plants primed by cowpea-volatiles, when they are subsequently attacked by *S. exigua*, should be strong, as they emitted high quantities of indole and (*Z*)-3-hexenyl acetate (Table S1).

Minor volatiles induced by differentially primed maize plants in response to *S. exigua* and *S. frugiperda* damage included geranyl acetate, methyl anthranilate, and *cis*-jasmone. An emission burst of these minor volatiles was observed after the maize plants were primed with HIPVs emitted from *S. exigua*-infested cowpea plants (Tables S1, S2, and S5). Various studies suggest that such minor volatiles can be particularly attractive to parasitoids and predators (D'Alessandro et al., 2009; Erb et al., 2015; Gouinguené et al., 2005; Köllner et al., 2010; Ton et al., 2007). Indeed, more work is required to understand the impact of minor volatiles on priming, and their role in direct and indirect defenses in maize.

To get further insight into how differentially priming of maize plants affects diurnal emission responses following subsequent herbivory, the HIPVs from maize plants were continuously measured by PTR-TOF-MS for 96 h (Fig. 5). This revealed that a maize plant primed with HIPVs of a cowpea plant released the highest bursts of HIPVs throughout the entire *S. exigua* infestations, especially after 24 h (Fig. 5). The measurements further confirmed that *S. frugiperda* infestation induces lesser quantities of VOCs (Fig. 5). In particular, *S. exigua* infestation of a maize plant exposed to pure air led to much higher emission rates of DMNT and indole at 24 h of infestation than a similarly treated plant infested with *S. frugiperda*. Furthermore, the maize plant primed by cowpea-HIPVs and then infested by *S. exigua* was associated with enhanced emissions bursts of DMNT, monoterpenes, sesquiterpenes and indole at 50 h, and TMTT at 75 h of infestation (Fig. 5D–H). This is likely associated with differential gene expression and substrate availability for the biosynthesis of terpenoids and indole on a time-dependent manner in a given maize replicate. In all cases, the emissions of constitutive and induced volatiles of maize plants, including (*Z*)-3-hexenyl acetate and terpenoids, followed a diurnal rhythm associated with higher emissions in the light and relatively lower emissions in the dark (Fig. 5B, and 5D-5G), as reported by (Gouinguené and Turlings, 2002). Based on their emission dynamics, these volatile signals can boost the attraction of natural enemies of herbivores, most of which are only active during the day (Turlings and Wäckers, 2004). In contrast, the GLVs (*Z*)-3-hexenal & (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, and indole were released irrespective of diurnal rhythm (Fig. 5A, 5C, and 5H), indicating that these VOCs can be highly useful for herbivore enemies, also at night. In addition, the remarkable spikes in release of different volatiles, particularly GLVs during light-to-dark transition in our study might be triggered by rapid changes in intracellular pH values, affecting membrane stability after fast light-to-dark transitions (Fig. 5A–D, and 5G); the exact mechanism for this phenomenon remains to be elucidated (Brilli et al., 2011; Jud et al., 2016).

Most reported cases on HIPVs affecting neighboring plants show priming effects rather than immediate defense induction (Turlings and Erb, 2018). This is explained by the fact that plants that directly launch their defenses upon "sensing" HIPVs released from neighboring plants may incur a fitness costs if they themselves are not attacked (Kessler et al., 2006). Hence, plants that refrain from allocating their limited resources to costly defenses prior to the arrival of herbivores, yet possess the ability to activate their defense metabolism faster and stronger upon herbivore attack may attain a fitness-benefit compared to plants that do not respond to the information conveyed by herbivore-induced plant volatiles (HIPVs) emitted by neighboring damaged plants (Kessler et al., 2006).

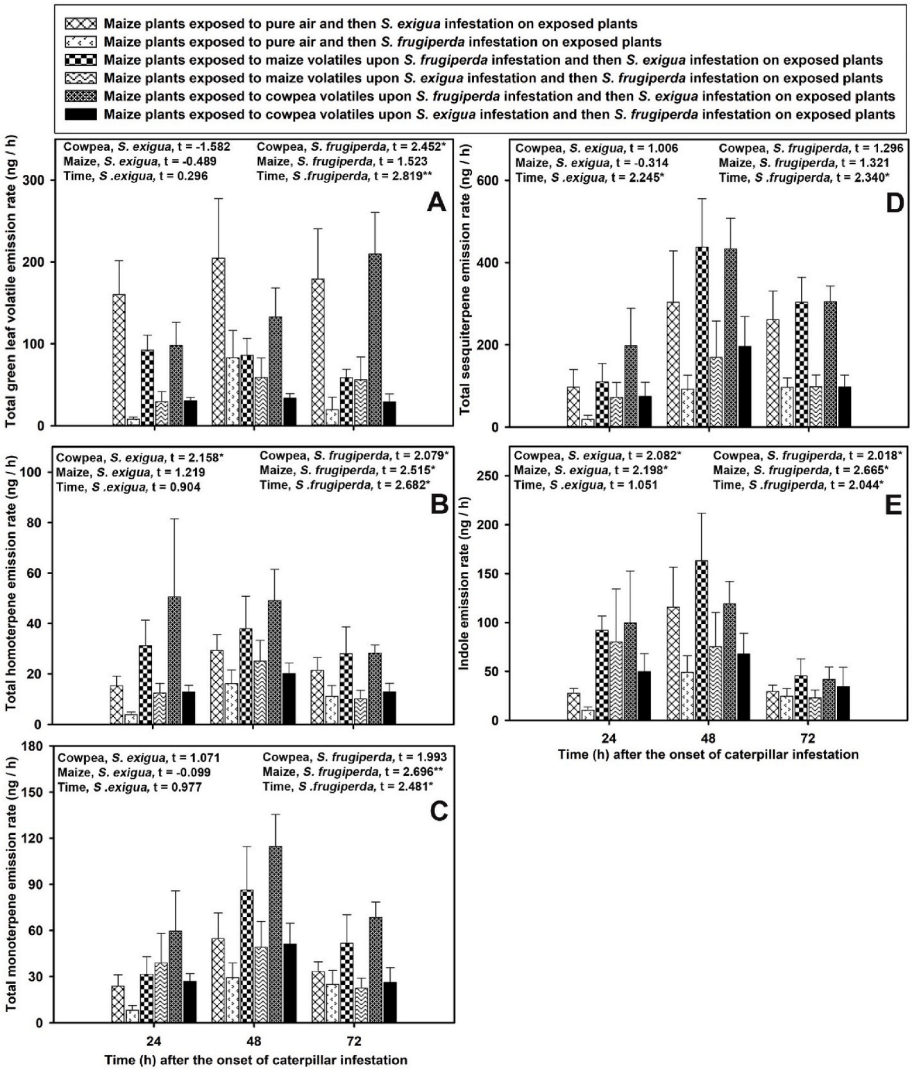

Fig. 4. Representative time-courses of emission rates (Mean + SE) of total GLVs (Fig. 4A), total homoterpenes (Fig. 4B), total monoterpenes (Fig. 4C), total sesquiterpenes (Fig. 4D), and indole (Fig. 4E) from differentially exposed-maize plants upon *S. exigua* and *S. frugiperda* infestations. The data presented for control plants are as in Figs. 2 and 3. There were six replicates for each treatment. Generalized linear models (GLM) fitted with gamma distribution were used to estimate individual and interactive effects of differential exposure and larval infestation time on HIPV emissions from differentially exposed-maize plants in response to *S. exigua* and *S. frugiperda* infestations. Data presentation as in S. Tables 3 and 4 The summary statistics of GLM as in Table 2.

It is commonly found that related plants respond more strongly to each other's volatiles, which is explained in the context of kin recognition and the exchange of honest information (Karban et al., 2013; Kessler et al., 2023). Here, we find that totally unrelated plants may also strongly respond to each other, possibly because of key signals that they share in common, leading to a strong eavesdropping effect (Karban et al., 2003). Follow-up work should focus on identifying such signals, also in other cropping systems. The outcome of this study implies that in intercropping the plant-to-plant signals could be exploited for crop protection and that species and varieties could be combined in a manner that their respective volatile signals have the optimal beneficial effect on pest resistance.

5. Conclusion

The present study demonstrates that maize plants are highly responsive to *S. exigua* infestations after being primed with HIPVs released from *S. exigua*-infested cowpea plants. The responsiveness was weaker for maize plants primed with HIPVs released from cowpea or maize plants in response to *S. frugiperda* infestation. Priming of maize plants with cowpea or maize volatiles could not overcome the distinctive volatile-suppression ability of *S. frugiperda*. However, further analysis of HIPV emissions using PTR-TOF-MS confirmed the stronger emissions by *S. exigua* caterpillars, which, in part, could also be explained by a marginally higher feeding rate on maize plants primed with HIPVs of *S. exigua*-infested cowpea plants. Further studies on how direct and indirect maize defenses are affected by differentially primed maize plants may expand our knowledge on the role of priming in plant-insect interactions and how it can be exploited for crop protection.

CRediT authorship contribution statement

Arooran Kanagendran: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Ted C.J. Turlings:** Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

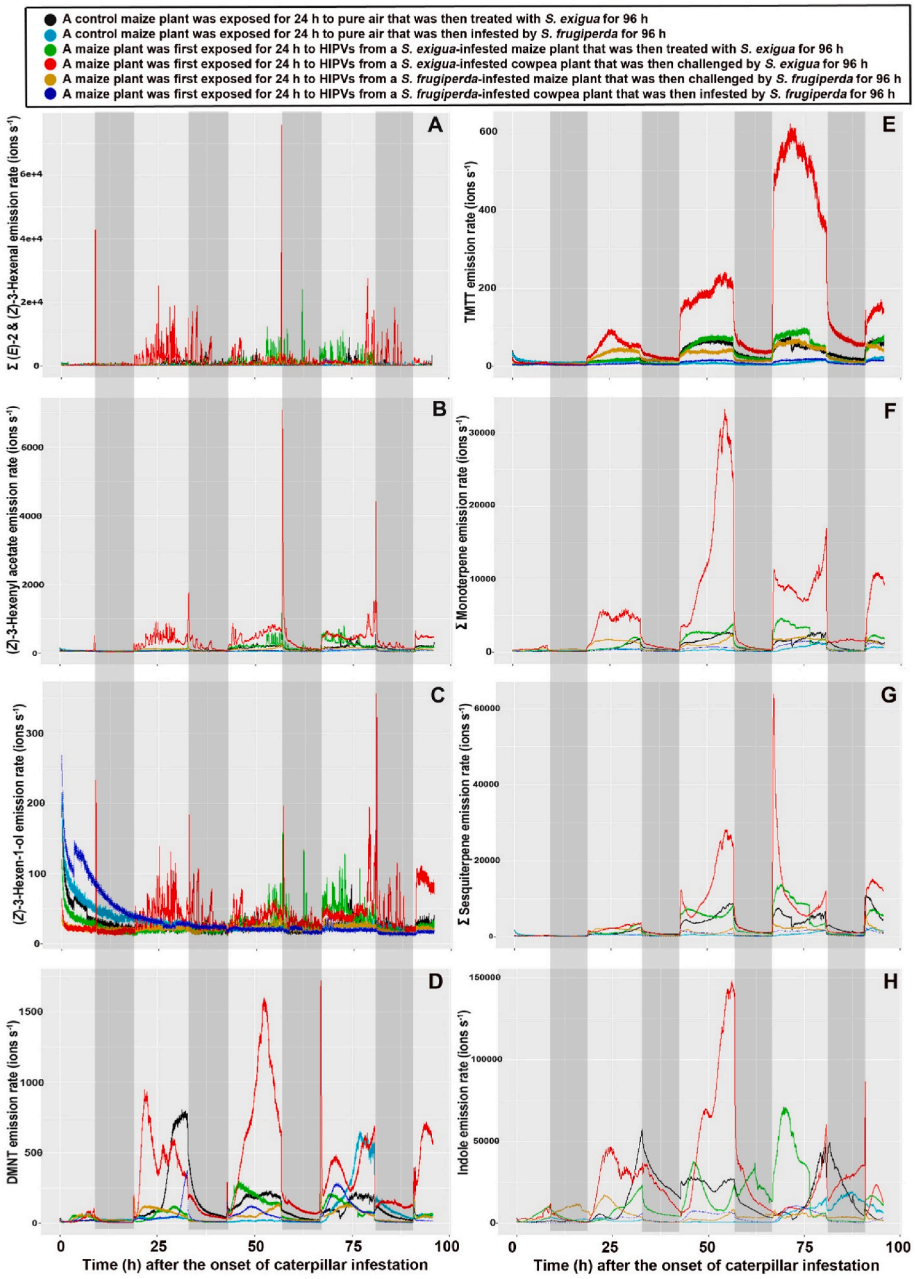

Fig. 5. Temporal emission of green leaf volatiles (GLVs) ∑ (*E*)-2-hexenal and (*Z*)-3-hexenal (A), (Z)-3-hexenyl acetate (B), (*Z*)-3-hexen-1-ol (C), DMNT (D), TMTT (E), ∑ monoterpenes (F), ∑ sesquiterpenes (G), and indole (H) released from maize plants exposed to pure air, maize volatiles, and cowpea volatiles. The HIPV emissions released from differentially exposed-maize plans upon *S. exigua* and *S. frugiperda* infestation were sampled with a proton-transfer reaction time-of-flight mass spectrometer (PTR-TOF-MS) for 96 h. In all cases, the sampling was started upon stabilization of air flows in the glass chambers, ca. 10 min after plant enclosure. The individual HIPVs were represented as their protonated parent ions: (*E*)-2-hexenal and (*Z*)-3-hexenal [*m/z* 99.080, $(C_6H_{10}O)H^+$], (*Z*)-3-hexen-1-ol [*m/z* 101.096, $(C_6H_{12}O)H^+$], (*Z*)-3-hexenyl acetate [*m/z* 143.106, $(C_8H_{14}O_2)H^+$], DMNT [*m/z* 151.148, $(C_{11}H_{18})H^+$], TMTT [*m/z* 219.210, $(C_{16}H_{26})H^+$], ∑ monoterpenes [*m/z* 137.132, $(C_{10}H_{16})H^+$] and [(β)-linalool *m/z* 155.143, $(C_{10}H_{18}O)H^+$], ∑ sesquiterpenes [*m/z* 205.195, $(C_{15}H_{24})H^+$] and [(*E*)-nerolidol *m/z* 223.205, $(C_{15}H_{26}O)H^+$], and indole [*m/z* 118.065, $(C_8H_7N)H^+$]. Data shown are of one replicate. In each plot, the light grey and dark grey areas represent light and dark period of a day respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Data availability

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jplph.2023.154164.

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