Priming of indirect defence responses in maize is shown to be genotype-specific

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Main conclusions The work provides an example of a genotype-specific priming effect in a crop plant.

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Abstract

Priming is an induced defence mechanism in which plants that have been exposed to elicitors, such as herbivore-induced plant volatiles (HIPVs), go into an alert state with faster and stronger responses against a future biotic challenge. This study evaluated whether HIPVs emitted by maize genotypes after herbivory by fall armyworm (*Spodoptera frugiperda*) larvae could prime neighbouring maize plants for an enhanced indirect defence response, and if priming was consistent across different genotypes. Two genotypes were selected based on their differences in HIPV emission: Sintético Spodoptera (SS), a relatively high emitter of HIPVs, and L3, a relatively low emitter of HIPVs. SS plants that were previously exposed to SS HIPVs initiated earlier and enhanced volatile production upon larval challenge, compared to SS plants that were previously exposed to SS undamaged plant volatiles. In addition, SS plants exposed to SS HIPVs and then to larval challenge attracted an egg parasitoid, *Telenomus remus*, at an earlier stage than SS plants that were only subjected to larval challenge, indicating a priming effect. There was no evidence of a priming response by L3 plants that were previously exposed to L3 or SS HIPVs. When comparing the gene expression of HIPV-exposed and undamaged plant volatile (UDV)-exposed plants, jasmonate-induced protein *GRMZM2G05154* and UDP-glucosyltransferase *bx8* genes related to the biosynthesis of DIBOA-Glu were upregulated. These data indicate that priming by HIPVs enhances indirect defence in maize plants as reported by other studies, and provide new information showing that the priming effect can be genotype-specific.

Key words plant-plant communication, natural enemies, plant defence, plant genotypes, *Spodoptera frugiperda*, volatiles compounds.

Introduction

Plant defence against insect herbivory can be triggered either directly by herbivores or indirectly through plant-to-plant communication. Priming is an induced defence mechanism in which plants that have been exposed to elicitors from biotic stress go into an alert state, with faster and stronger responses against a future biotic challenge (Dicke et al. 1990; Bruin et al. 1992; Bruin and Dicke 2001; Dicke and Bruin 2001; Bruce and Pickett 2007). Studies on priming of plant defence can potentially provide new insights into plant-to-plant

communication and underpin the development of new tools for crop protection based on inducible defence mechanisms that have reduced biological costs compared to metabolically expensive constitutive defence mechanisms (Kessler et al. 2006; Hilker et al. 2016; Vries et al. 2016; Mauch-Mani et al. 2017). Priming in plants can be activated by herbivore-induced plant volatiles (HIPVs) that are released following feeding by either generalist herbivores such as *Spodoptera littoralis* (Lepidoptera: Noctuidae) (Ton et al. 2007) or specialists such as *Mythimna separata* (Lepidoptera: Noctuidae) (Ramadam et al. 2011); egg deposition by *Chilo partellus* (Lepidoptera: Crambidae) (Mutyambai et al. 2016); biological secretions such as the regurgitant of *Spodoptera exigua* (Lepidoptera: Noctuidae) (Engelberth et al. 2004); the application of synthetic volatile compounds such as green leaf volatiles (Engelberth et al. 2007); the phytopheromone *cis*-jasmone (Oluwafemi et al. 2013); the peptide phytohormone systemin in tomato plants (Coppola et al. 2007); the plant volatile compound indole which primes defence in different plant species such as maize, cotton (Erb et al. 2015) and rice (Ye et al., 2019). Priming effects are observed through changes in volatile and non-volatile production (Erb et al. 2015; Hu et al. 2018), by enhanced indirect (Ton et al. 2007) and direct defence (Hu et al. 2018), and by down- and up-regulation of defence-related genes (Ton et al. 2007; Engelberth al. 2007; Hu et al. 2018; Ye et al. 2019).

Maize is an important crop for food security in several countries with low incomes, including Brazil (Wu and Guclu 2013; Prasanna 2014). The fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae), is an important maize pest in Brazil (Cruz 1995; Cruz et al. 2010) that is usually controlled by heavy pesticide applications (Toscano et al. 2012). In the last 10 years, *Bt* technologies have contributed to FAW control, but recent studies have shown that *Bt* maize does not control FAW populations in several regions of Brazil, with populations having become resistant to *Bt* plants (Faria et al. 2014; Bernardi et al. 2015). Therefore, alternative control strategies are necessary to develop more sustainable maize cropping systems, mainly aiming to help smallholder farmers that in Brazil are responsible for 30% of maize production. The recruitment of biological control agents such as natural enemies through deployment of sentinel and smart plants is considered as a promising alternative strategy for integrated pest management (Pickett and Khan 2016). Sentinel plants were first defined as plants that are susceptible to herbivore attack, pathogen infestation, and other stresses, emitting signalling related to this stress earlier than resistant plants. Sentinel and smart plants have been recently defined as genetically modified or selected varieties that present traits allowing them to respond earlier to stress, therefore signalling to neighbouring plants regarding

impending danger (Pickett and Khan 2016). Sentinel plants, for example, by acting as a source of HIPVs, could prime neighbouring plants, thereby enhancing their defence by attracting natural enemies. Smart plants can also be defined as plants that are able to perceive HIPVs more efficiently than non-smart plants, for example, and get prepared, i.e., primed, for future attack (Pickett and Khan 2016).

The egg parasitoid, *Telenomus remus* (Hymenoptera: Platygastridae) has a high potential for parasitism against several *Spodoptera* spp., mainly *S. frugiperda*. Despite the fact that *T. remus* has not established in Brazilian fields, several studies have shown that it has high parasitism potential with inundative release (Figueiredo et al. 1999, 2002; Pomari et al. 2013). In addition, *T. remus* is known to be attracted to HIPVs emitted by maize plants (Peñaflor et al. 2011; Michereff et al. 2019). Our previous work showed that maize genotypes produce different levels of HIPVs in response to the feeding damage by 2nd instar larvae of *S. frugiperda*. A Sintético Spodoptera (SS) genotype was shown to be a relatively high emitter of HIPVs, showing indirect defence activation by *S. frugiperda* herbivory damage, through *T. remus* attraction (Michereff et al. 2019), whereas L3 genotype, that was shown to be a relatively low emitter of HIPVs and did not attract the egg parasitoid, did not have its indirect defence activated by *S. frugiperda* herbivory (Michereff et al. 2019).

Based on earlier work (Ton et al. 2007; Ramadam et al. 2011), HIPV-exposed maize would be expected to express a stronger and faster indirect defence response to FAW damage. However, there is no information on whether or not HIPVs emitted by different maize genotypes are capable of delivering the priming effect. Therefore, this study investigated whether or not HIPVs emitted by two maize genotypes, SS and L3; that differ in their HIPVs emissions; could both prime neighbouring plants for a faster defence response, and whether or not the defence of HIPV-primed plants was more enhanced compared to the defence of naïve maize plants subjected only to FAW damage. To address these questions, the response of *T. remus* to HIPVs emitted by HIPV-exposed, UDV-exposed and FAW-damaged plants was evaluated, and differential gene expression of HIPV-exposed and UDV-exposed plants was investigated.

Materials and methods

Insect rearing

Spodoptera frugiperda and *Telemonus remus* were maintained in separate environmental rooms at 27±1 °C, with 65±10% relative humidity and a 14 h photoperiod. *S. frugiperda* larvae were obtained from a laboratory colony maintained at Embrapa Genetic Resources and Biotechnology in Brasília, DF, Brazil. The larvae were reared in plastic containers on an artificial diet based on beans (*Phaseolus vulgaris*). Second instar larvae (Schmidt et al. 2001) were used in experiments and starved for 24 h before the experiment. *T. remus* was obtained from a laboratory colony raised on *S. frugiperda* eggs. The wasps were maintained in acrylic cages (75 cm^2 angled neck tissue culture flasks; ICN Biomedicals, Irvine, CA, USA) and fed with a drop of honey. Following hatching, the parasitoids were kept in acrylic cages for 24 h for mating. Two-day-old females with oviposition experience were used in the experiments (Michereff et al. 2019). As showed by Peñaflor et al (2011), experienced *T. remus* females respond better to herbivo**r**e-induced plant volatiles (HIPVs) than naïve females; so for conditioning, 10 parasitoids were placed into a 10 L glass chamber with 100 eggs of *S. frugiperda* laid in a filter paper, and with HIPVs emitted from maize plants. The source of the HIPVs was a single maize plant that was placed into another 10 L glass chamber with five second instar *S. frugiperda* larvae for 24 h. The chamber with the maize plant releasing HIPVs was connected by Teflon tubing to the chamber with the egg parasitoids, and the airflow from the chamber releasing the HIPVs was pulled, using an air pump, to the glass chamber with the egg parasitoids at a flow of 0.6 L/min. When the egg parasitoid started to forage for eggs, they were removed and used in behaviour assays the following day. Parasitoids were observed for a maximum of 1 h, but for the most part, parasitoids started foraging after 2 minutes.

Plants

Maize seeds were obtained from the Germplasm Bank of Embrapa Maize and Sorghum in Sete Lagoas, MG, Brazil (19°27′57″S and 44°14′48″W) and germinated on damp paper. The genotypes used were Sintético Spodoptera (SS) and L3. The SS genotype has an antixenotic resistant effect to *Spodoptera frugiperda* developed from elite materials *(*MIRT do CIMMYT e CMS 23 (Antigua vs República Dominicana), and this genotype was not registered yet. L3 genotype was registered in Brazilian Agriculture ministry as CMS-27, it is a susceptible genotype (Silveira et al. 1997; Viana and Potenza 2000; Costa et al. 2006). Accession data for L3 genotype is available in the Alelo germplasm bank (Alelo, 2020). After 4 days, the seeds were transplanted to pots with a mixture of soil and organic substrate (in a proportion of 1:1

w/w) and kept in a greenhouse (14 h photoperiod). The plants used in the experiments were grown for 9-10 days after emergence and had three fully expanded leaves.

Plant-to-plant communication experiments

Plant-to-plant communication experiments were set up and divided into three phases (see Fig. S1 for schematic representation, Supplementary Material):

Phase 1 - source plants (SP) releasing HIPVs or undamaged plant volatiles (UDVs) were prepared. For this, three maize plants were placed into cylindrical glass chambers (internal volume 10 L). The experimental plants were either those that received five second instar larvae of *S. frugiperda* (HIPV emission plants) or those that did not receive *S. frugiperda* larvae (UDV emission plants) (N = 6 per treatment). In a previous study, it was shown that maize plants produce a higher level of HIPVs after 6 h of herbivory damage (Michereff et al. 2019). Therefore, the duration of phase 1 was 6 h.

Phase 2 - the glass chambers with three SP plants were connected to other glass chambers containing three target plants (TP) to start phase 2. TP received either HIPVs or UDVs for 24 h. After this time, the chambers were disconnected, and the TP were allowed to rest for 1 h before being transferred to new glass chambers.

Phase 3 - TP that were treated in phase 2 with HIPVs or UDVs received one of the following treatments: 1) five larvae of *S. frugiperda* (HIPVs + Sf or UDVs + Sf) (N = 6 for each treatment) or 2) no further challenge (HIPVs - Sf or UDVs - Sf) (N = 6 for each treatment). Plant volatiles under these four treatments were collected at 0-2, 2-4, 4-8, 8-16 and 16-24 h. To minimize contamination by volatiles from the soil, the pots were wrapped in aluminium foil.

In summary, the following treatments were obtained:

HIPVs + Sf: TP that received SP HIPVs in phase 2 and received larvae of *S. frugiperda* in phase 3;

HIPVs - Sf: TP that received SP HIPVs in phase 2 and had no further biotic challenge in phase 3;

UDVs + Sf: TP that received SP UDVs in phase 2 and were subjected to herbivory in phase 3

UDVs - Sf: TP that received SP UDVs in phase 2 and had no further biotic challenge in phase 3.

SS and L3 plant genotypes were exposed to HIPVs and UDVs from the same genotype. L3 plants were also exposed to HIPVs and UDVs emitted by SS plants, since earlier work (Michereff et al. 2019) showed that SS plants released a higher level of HIPVs compared to L3 plants.

Collection of UDVs and HIPVs

Volatile collection from plants under the four treatments (HIPVs + Sf, HIPVs - Sf, UDVs + Sf, UDV - Sf) were collected at 0-2, 2-4, 4-8, 8-16 and 16-24 h after the infestations were initiated during phase 3 (N = 6 replicates for each time and genotype) (Fig. 1).

To determine the chemical profile of volatiles of undamaged and herbivory-damaged maize plants, three undamaged (-Sf) and *S. frugiperda*-damaged (+Sf) plants were placed in cylindrical glass chambers (internal volume 10 L), and the volatiles were collected from the same individual plant for 4-8 h and 8-16 h after the infestations were initiated (N = 6 replicates for each time and genotype). These times were selected based on previous work (Michereff et al., 2019), which showed that plants start to significantly enhance volatile production after 6 h of herbivory compared to that for undamaged plants.

For all treatments, volatiles were collected in glass tubes containing the adsorbent Porapak Q (100 mg, 80-100 mesh) that were connected via a PTFE tube to a vacuum pump at a flow of 0.6 L/min, and the air entrance was connected to an activated charcoal (1.0 L/min) air flow, creating a positive push-pull system (Moraes et al. 2008). The trapped volatiles were eluted from the adsorbent using 500 µL of n-hexane and concentrated to 50 µL under a N_2 flow. Extracts were stored at -20 °C until analyses by coupled gas chromatography flame ionization detector (GC-FID) and coupled GC mass spectrometry (GC-MS). For qualitative analysis, selected extracts were analysed using GC (Agilent GC7890A, USA) coupled to a mass spectrometer (Agilent 5975MSD, USA) equipped with a quadrupole analyser, a nonpolar DB-5MS column (30 m × 0.25 mm ID and 0.25 µm film thickness; J&W Scientific, Folsom, CA, USA), and a splitless injector with helium as the carrier gas. Ionization was by electron impact (70 eV and source temperature 200 °C). Data were collected and analysed with GC-MS ChemStation 2.1 Software (Agilent, California, USA). Volatile compounds in the extracts were identified by comparing spectra with library databases (NIST 2008) or published spectra and confirmed using authentic standards when available. For quantitative analyses, the volatiles of all treatments were analysed by GC-FID (Agilent 7890A, DB-5MS) using a 30 m x 0.25 mm

ID column (0.25 µm film thickness, J&W Scientific, Folsom, CA, USA). The temperature program was 50 °C (2 min), 5 °C/min to 180 °C (0.1 min), and 10 °C/min to 250 °C (20 min). The column effluent was analysed with a FID at 270 °C. One microliter of 16-hexadecanolide was added as an internal standard (IS) with a final concentration of 9.8 µg/mL. The response factor for all compounds was considered 1. Two microliters of each sample were injected using the splitless mode with helium as the carrier gas. The amounts of volatile chemicals released by the plants at different times were calculated in relation to the area of the internal standard. Data were collected with EZChrom Elite software (Agilent, California, USA) and were recorded using Excel (Microsoft Corporation, 2007). The absolute configuration of linalool released by SS and L3 maize genotypes was determined in our previous study as 1:1(*R*)- and (*S*)-linalool (Michereff et al. 2019).

Chemicals

n-Hexane (95%, suitable for pesticide residue analysis), Porapak Q, indole (99%), α-camphene (95%), (*E*)-(1*R*,9*S*)-caryophyllene (98%), myrcene (95%), α-humulene (96%), geranylacetone (97%), ocimene (mixture of isomers, > 90%) and geranyl acetate (97%), cyclosativene (99%) were purchased from Sigma-Aldrich (Steinheim, Germany). (*E*)-2-Hexenal (95%) and (*Z*)-3-hexen-1-ol (98%) were purchased from Sigma-Aldrich (Gillingham, Dorset, UK). (*E*)-β-Farnesene (98%) was provided by Shin-Estu (Japan). (*Z*)-3-Hexenyl acetate (98%) was purchased from Alfa Aesar (Heysham, UK). (*E*)-2-Hexenyl acetate (97%) and linalool were purchased from TCI America (Portland, USA). (*E*)-4,8-Dimethyl-1,3,7-nonatriene (DMNT) and (*E*,*E*)-4,8,12-trimethyl-1-,3,7,11-tridecatetraene (TMTT) were synthesized from geraniol and (*E*,*E*)-farnesol, respectively (Leopold 1990).

Bioassays

Y-Tube olfactometer bioassays were conducted with the egg parasitoid *Telenomus remus* to determine whether or not SS and L3 plants previously exposed to SS HIPVs and subsequent herbivory damage by *S. frugiperda* had their defence enhanced, compared to plants that were subjected to herbivory damage of *S. frugiperda* without previous exposure to HIPVs. The olfactometer consisted of square acrylic blocks (19 × 19 cm) with a 1 cm Y-shaped cavity sandwiched between two glass plates (Moraes et al. 2008). The leg of the cavity was 8 cm long,

and each arm was 7 cm long. Air that was charcoal-filtered and humidified was pushed through the system at 0.6 L/min and pulled out at 0.2 L/min by a push-pull system. A single *T. remus* female was introduced at the base of the Y-tube and observed for 600 s. The first-choice arm, which was the first one that the wasp entered and remained in for at least 30 s, and the residence time, which was the amount of time the parasitoid remained in each arm, were recorded. After every five repetitions, the plants were replaced, and the positions of the arms of the olfactometer were changed to avoid bias in the parasitoid responses.

A previous study reported that *T. remus* responded to HIPVs induced by *S. frugiperda* within 24 h of damage (Michereff et al. 2019). In this study, the response of parasitoids to HIPVs released by naïve maize plants was evaluated at 8 and 16 h after *S. frugiperda* herbivory damage and to HIPVs emitted by primed plants (HIPVs + Sf) at 8 and 16 h after herbivory. Each female was used only once, and 40 repetitions were conducted for the following treatment combinations with the volatiles emitted from 1) HIPV-exposed plants and treated with *S. frugiperda* larvae (HIPVs + Sf), 8h, vs. air; 2) HIPVs + Sf, 16h, vs. air 3) UDVs + Sf, 8h, vs. air; 4) UDVs + Sf, 16h, vs. air; 5) HIPVs + Sf vs. UDVs - Sf 8 h; 6) HIPVs + Sf vs. UDVs - Sf 16 h; 7) HIPVs + Sf vs. UDVs + Sf 8 h; 8) HIPVs + Sf vs. UDVs + Sf 16 h; 9) + Sf vs. - Sf 8h and 10) + Sf vs. - Sf 16h. Treated and untreated plants were placed in glass chambers (10 L) and connected to the olfactometer via silicone tubing. To avoid possible chemical signalling between plants, *S. frugiperda* herbivory-damaged and undamaged plants were kept in different rooms under the same temperature, humidity, and lighting conditions (26 ± 1 °C and 65±10% r.h. under a photoperiod of 14L:10D). All bioassays were conducted from 10:00 to 18:00 h. As the chemical profile from L3 plants previously exposed to L3 HIPVs did not show any difference between treatments, therefore they were not tested.

RNA isolation and evaluation

The aerial parts of maize plants that were exposed to HIPVs or UDVs in phase 2 for 2 or 24 h were used for RNA isolation. Each treatment was repeated once and consisted of a pool of three biological replicates. Plants were ground to a fine powder in liquid nitrogen using a sterile mortar and pestle. Total RNA was extracted from 100 mg of powdered frozen maize leaves with Trizol regent. To eliminate possible DNA contamination, 10 µg of total RNA was treated with 6 U of amplification grade DNAse I (Invitrogen) in 1X DNAse I reaction buffer (Invitrogen). DNase I was inactivated, followed by purification.

RNA-Seq library construction and sequencing

Samples were analysed with the 2100 Bioanalyzer (Agilent Technologies) for quality control and quantification. Only samples with high scores of RNA integrity (RIN > 7) were further processed. RNA transcriptome sequencing was performed using an Illumina HiSeq4000.

Sequencing reads analysis

The raw data were processed using the Trimmomatic (V. 3) program to eliminate low-quality sequences (FastQC < 30) and trim out the adapters (Bolger et al., 2014). High-quality clean sequences were mapped into the *Zea mays* genome (V.AGPv3.22) using the TopHat2 program (Kim et al., 2013). Read counts were calculated using htseq-count (V. 0.6.1p1) with the following parameters: -r pos -t gene -m union -i ID -f bam (Anders et al., 2014). Differentially expressed genes were assessed using EdgeR and RVUSeq Bioconductor packages (Robinson et al., 2010, Risso et al., 2014). Samples were compared according to the following: 1) Treatment effect: HIPV and UDV-exposed plants at 2 h and 24 h; 2) Time + treatment effect: HIPV-exposed plants 2 h vs 24 h and UDV-exposed plants 2 h vs 24 h. Cut-off values were set up as FDR < 0.05 and absolute fold-change value above 2.

PFAM annotation and gene ontology analysis

The assembled transcripts were annotated with PFAM terms (Pfam30.0). The pfam2go table (Mitchell et al. 2015) was used to annotate the maize transcripts with Gene Ontology (GO) terms. A hypergeometric test within FUNC (Prüfer et al. 2007) was applied to identify enriched GO terms on differentially expressed genes (DEGs). REVIGO (http://revigo.irb.hr/) was applied to remove redundant terms. GO enrichment factor was calculated as the ratio between the number of observed and expected genes in relation to the total number of genes in the sample or genome, respectively, of each significative term. KEGG enrichment analysis and metabolic pathway enrichment analysis for DEGs were predicted using String App for Cytoscape v.3.6.1 with the following parameters: medium confidence (0.400) for treatment effect data, high confidence (0.700) for time + treatment effect, and hide disconnected nodes

in the network. KEGG enrichment analysis was calculated by String Enrichment App for Cytoscape.

Statistical analysis

To evaluate the effect of an individual volatile compound, the data were subjected to a repeated measurement with a linear mixed model (LMM) fitted by maximum likelihood. If the individual compound did not show a significant effect, the statistical GLM was applied using a gamma distribution and an inverse link function. If the GLM showed significant differences, the data were subjected to contrast analysis. For LMM, a simultaneous Dunnett contrast test was applied for general linear hypotheses with multiple comparisons of means. The change in the chemical profile of maize plants subjected to different treatments (UDVs + Sf, UDVs - Sf, HIPVs + Sf, and HIPVs - Sf) over time was assessed using principal response curve (PRC) analysis (van den Brink and ter Braak 1999; Michereff et al. 2011). This multivariate technique allows the assessment of repeated measurements over time, focusing on the proportion of variance explained by the treatments and the time compared to the control (undamaged plants). In each set of analyses, the significance was determined by a Monte Carlo permutation test. All analyses were performed using the statistical program R 3.3.2 (R core team).

To evaluate the influence of the compounds used in the bioassays (HIPVs + Sf, HIPVs and UDVs) at specific time-points (4-8 and 8-16 h), a principal component analysis (PCA) was applied to the data. The PCA was performed using a variance-covariance matrix and comparisons between and within groups using paleontological statistics software (PAST version 3.10). The data from bioassays were first tested to evaluate the influence of the individuals (plants) using a repeated measure with binomial distribution. Then, the first-choice responses of the egg parasitoid to each treatment in the Y-tube olfactometer bioassays were analysed using logistic regressions to estimate the probability of each choice. The model concurred with the side (left or right) on which the test odour was presented. The hypothesis of no preference (i.e., the proportion of choosing each odour = 0.5) was tested by the chi-square Wald test. The data for the residence times of the egg parasitoid in each olfactometer arm were analysed by paired *t*-tests. If insects did not move after 3 min, they were considered non-responsive and were not included in the statistical analysis. All analyses were performed using the statistical program R 3.3.2 (R core team).

Results

Chemical analysis of volatiles

To evaluate whether or not HIPVs emitted by maize genotypes following *S. frugiperda* larval herbivory could result in a faster and enhanced response in neighbouring maize plants of the same genotype, volatiles emitted by SS and L3 genotypes after exposure to four different treatments were collected and compared (Supplementary Fig. S2, SS genotype, and Fig. S3, L3 genotype). The total amount of volatiles released by SS maize in phase 3 following exposure to SS maize HIPVs and UDVs in phase 2 was different between the treatments over time; plants that received either HIPVs or UDVs followed by *S. frugiperda* larvae (HIPVs + Sf and UDVs + Sf) produced higher amounts of volatiles during 4-8, 8-16 and 16-24 h time periods compared to plants that only received either HIPVs (HIPVs - Sf) or UDVs (UDVs - Sf) (Fig. 1a, Supplementary Table S1). SS maize plants that were exposed to SS HIPVs and were treated with *S. frugiperda* larvae (HIPVs + Sf) produced higher levels of volatiles during 8-16 h compared to all other treatments (Fig. 1a). By contrast, the total amount of volatiles released by L3 maize plants exposed to L3 HIPVs and UDVs did not differ between the treatments (Fig. 1b, Supplementary Table S1). When L3 plants were exposed to SS HIPVs and UDVs, differences in the amount of volatiles released were observed 2-4, 8-16 and 16-24 h after treatment was initiated in phase 3 (Fig. 1c, Supplementary Table S1). However, the volatiles released by the UDV + Sf and HIPVs + Sf treatments were not different (Fig. 1c, Supplementary Table S1).

Plant volatiles cannot be considered as independent variables because they can have common precursors or enzymes, and their quantities can be related to the quantity of precursor or enzyme involved in their biosynthesis (Hare 2011). Therefore, to determine if priming of volatile production was occurring in phase 3, temporal changes in the chemical profile of volatiles emitted from plants exposed to HIPVs + Sf, HIPVs - Sf, UDVs + Sf and UDVs - Sf treatments in phase 3 were assessed using Principal Response Curve (PRC) multivariate analysis, with the amounts of each volatile compound being used to build curves for the different treatments (Michereff et al. 2011). In a PRC plot, when the curves are closer, this indicates higher similarity between treatments. The UDVs - Sf treatment was used as the base response and the amount of each compound in the other three treatments was compared to this treatment.

For the SS maize genotype, PRC analysis showed consistent variability between treatments over time, and the treatments were different (Monte Carlo permutation test F = 20.90, P = 0.001) (Fig. 2a, Supplementary Table S2). From the total variance in the blend composition of volatiles released, 5.0 % was explained by sampling time, and 27.4 % was explained by the treatment. The main compounds responsible for differences between the treatments were identified using the weight-value, of which values higher than |1.0| was a significant contribution of the compound to the accomplishment of the PRC curves. Thus, (*Z*)-3-hexenyl acetate, (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), (*E*)-2-hexenal, indole, (*R,S*)-linalool, cyclosativene, myrcene, (*E*)-β-farnesene, (*E*)-(1*R*,9*S*)-caryophyllene, (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) and (*E*)-ocimene were the main compounds that contributed to the difference between treatments (Fig. 2a). The curves of the HIPVs + Sf and HIPVs - Sf treatments were closer at the 0-2h time period evaluated. However, for the time periods thereafter the two curves became more distant, which means that this induction was not persistent. By contrast, the curve of HIPVs + Sf after 4-8 h became more distant from all other curves, indicating higher volatile production compared to other treatments. For the 16-24h time period, HIPVs + Sf and UDVs + Sf curves became closer, as expected, with both plants during this time producing high levels of volatiles due to *S. frugiperda* herbivory damage. GLM analysis showed that for HIPVs + Sf, (*E*)-ocimene, DMNT and (*E*)-β-farnesene were produced in higher amounts compared to all other treatments during the 8-16 h time period (Supplementary Fig. S2 and Table S3). In addition, GLM analysis showed that the compounds (*Z*)-3-hexenyl acetate, indole, (*E*)-β-farnesene, DMNT and (*E*)-ocimene were produced in higher amounts for HIPVs + Sf compared to all other treatments during the 16-24 h time period, and (*E*)-2-hexenal, (*RS*)-linalool and (*E*)-(1*R*,9*S*)-caryophyllene were induced by UDV+Sf, HIPVs + Sf and HIPVs-Sf in earlier time periods (Supplementary Fig. S2 and Table S3). Therefore, the significant compounds in the different time periods reported by the GLM and the PRC analysis are the same (Fig 2a, Supplementary Fig. S2 and Table S3), except for cyclosativene which was not significant in univariate (GLM) analysis, but was significant in the multivariate (PRC) analysis.

For the L3 maize genotype, comparison of the blend of volatiles emitted by UDVs - Sf plants with those of the other treatments showed that the variance exhibited in the first PRC axis was not significant (Monte Carlo permutation test F = 4.95, P = 0.61) (Fig. 2b, Supplementary Table S2). For all time periods evaluated, analysis did not show any significant

difference between treatments, indicating that the blends of volatiles from the four treatments were similar (Fig. 2b, Supplementary Table S2).

When evaluating the effect of SS HIPVs and UDVs on the L3 genotype, PRC analysis showed a consistent variability over time between treatments, and the treatments were different (Monte Carlo permutation test $F = 26.133$, $P < 0.001$) (Fig. 3c, Supplementary Table S2). From the total variance in the blend composition of volatiles released, 22.0 % was explained by sampling time, and 36.4 % was explained by the treatment. The main compounds responsible for differences between the treatments were (Z)-3-hexen-1-ol, (*Z*)-3-hexenyl acetate, DMNT and (*E*)-β-farnesene (Fig. 2c, Supplementary Fig. S4). The curves of the HIPVs + Sf, UDVs + Sf and HIPVs - Sf treatments were closer during the 0-2 h time period, as for the treatments in Fig. 3a. However, for the time periods thereafter, the curve of HIPVs - Sf became more distant from HIPVs + Sf and UDVs + Sf, and became closer to the UDVs - Sf curve, suggesting that this induction was not persistent.

The volatile chemical profile of the treatments used in Y-tube olfactometer bioassays to the genotype SS (see below), were analysed using a principal component analysis (PCA). The chemical profiles of plants that were not exposed to volatiles compounds, undamaged maize plants (-Sf) and maize plants that received *S. frugiperda* larvae (+Sf) were grouped and clearly separated from maize plants exposed to HIPVs that received *S. frugiperda* larvae (HIPVs + Sf) at both time-points evaluated (8h and 16h after treatments) (Fig. 3a and 3b).

Natural enemy behaviour

Y-Tube olfactometer bioassays were conducted with the egg parasitoid *Telenomus remus* to determine whether or not maize plants that were exposed to treatments in phase 3 were primed for a faster defence response. Bioassays were conducted with volatiles emitted by the SS and L3 genotypes after they were exposed to HIPVs and UDVs from SS genotype.

When the volatiles of SS maize plants treated with HIPVs + Sf was compared to air, the parasitoids significantly preferred the volatiles from HIPVs + Sf treated plants at both time points (8h; $\chi^2 = 4.69$, $P = 0.012$, 16h; $\chi^2 = 6.03$, $P = 0.031$) (Fig. 4, entries 1-2). Conversely, when the volatiles emitted by UDVs + Sf was compared to air, there was no significant difference (8h; $\chi^2 = 0$, $P = 1.0$, 16h; $\chi^2 = 0.09$, $P = 0.752$) (Fig. 4, entries 3-4). In dual-choice experiments, when the volatiles of HIPVs + Sf treated plants was compared to the volatiles of

UDVs - Sf plants, the parasitoids significantly preferred the volatiles from HIPVs + Sf treated plants at both time points (8h; $\chi^2 = 4.937$, $P = 0.026$, 16h; $\chi^2 = 3.814$, $P = 0.05$) (Fig. 4, entries 5-6). However, when the volatiles emitted by HIPVs + Sf plants was compared with the volatiles emitted by UDVs + Sf plants, there was no significant difference *(*8h; $\chi^2 = 0.079$, $P = 0.777$, 16 h, $\chi^2 = 0.079$, $P = 0.777$) (Fig. 4, entries 7-8). Furthermore, the parasitoids were unable to choose between volatiles from +Sf treated plants and -Sf treated plants (8h; $\chi^2 = 0.398$, $P = 0.527$, 16h; $\chi^2 = 6.04\text{x}10^{-34}$, $P = 1$) (Fig. 4, entries 9-10).

When evaluating the amount of time that the parasitoids spent in each arm of the olfactometer, the parasitoid spent more time in the olfactometer arm with the volatiles emitted by HIPVs + Sf compared to air at both time points evaluated 8h ($t = 3.314$, df = 39, $P = 0.002$, entry 1) and 16 h ($t = 3.602$, df = 39, $P < 0.001$, entry 2) (Fig. 5). When volatiles emitted by UDVs + Sf plants at 8 h and 16 h after herbivory were evaluated against air at 8h and 16 h, there was no significant difference in residence time between arms (0-8 h: : $t = 1.271$, df = 39, $P < 0.211$ and 0-16h : $t = 1.351$, df = 39, $P = 0.184$, entries 3-4) (Fig. 5). The parasitoid spent more time in the arm with the volatiles emitted by HIPVs + Sf plants compared with the arm containing UDVs + Sf at 16 h ($t = 2.285$, df = 39, $P = 0.026$, entry 6). However, when the same treatments were evaluated at the 8 h time-point, there was no significant difference in the amount of the time spent in the arms of the olfactometer ($t = 1.812$, df = 39, $P = 0.076$, entry 5). The same was observed when volatiles emitted by HIPVs + Sf plants at 8 h and 16 h after herbivory were evaluated against volatiles emitted by UDVs + Sf plants at 8 and 16 h (0-8 h: $t = 573$, df = 39, $P = 0.570$ and 0-16 h: $t = 1.481$, df = 39, $P = 0.145$, entries 7-8). *T. remus* also spent the same time in the arms of the olfactometer when +Sf were compared with constitutive plant volatiles (-Sf) at 8 h ($t = -0.263$, df = 3, $P = 0.794$) and at 16 h ($t = -0.747$, df = 39, $P = 0.459$), entries 9-10 (Fig. 5).

When the volatiles from L3 that was exposed to SS HIPVs and UDVs were tested in Y olfactometer bioassays, the parasitoids did not respond to any of the treatments evaluated and also the residence time was not different between treatments (Supplementary Figs. S5a and b).

RNA-seq analysis – Treatment and treatment + time effect

A total of 2,394 and 3,099 genes were found to be differentially expressed (DE) between HIPV- and UDV-exposed SS maize plants, considering both 2 and 24 h time points. Of these DE genes (DEGs), 1,255 genes were common between the HIPV- and UDV-exposed

plants (Supplementary Fig. S6). Eleven genes were upregulated in the UDV-exposed plants but downregulated in the HIPV-exposed plants, and only four genes were downregulated in the UDV-exposed plants but upregulated in the HIPV-exposed plants (Supplementary Fig. S6, Panel b). The transcription levels of plant defence genes in the leaves of non-infested HIPV exposed plants, and genes related to plant defence against herbivores, such as Bowman-Birk-type trypsin inhibitor (TI), were downregulated when compared with the transcription levels of UDV-exposed plants at 2 and 24 h after treatment and when compared the same treatment with itself at the different time-points evaluated, i.e., 2 h and 24 h. In contrast, other plant defence genes were upregulated when the comparison was made between the UDV- and HIPV-exposed plants after 2h and 24 h of treatment, with the *WRKY74*-superfamily *GRMZM2G163418* of TFs and *GRMZM2G170338* defence related gene and jasmonate-induced protein *GRMZM2G05154* being upregulated in HIPV-exposed plants after 24 h. When comparing the gene expression of HIPV-exposed plants at 2h and 24h, the jasmonate-induced protein and DIBOA UDP-glucosyltransferase *bx8* genes were upregulated in HIPV-exposed plants at 24 h (Supplementary Fig. S6).

Expression analysis of HIPV- and UDV-exposed plants

DEG functional annotation and subsequent data mining were based on the Gene Ontology (GO) vocabulary after PFAM terms annotation. The GO terms during the first 2 h of plants exposed to HIPVs (red bubbles, Fig. 6) are related to important cellular components (CC), such as chloroplasts and other plastids that are involved in the activation of plant defence mechanisms. During this time, there were no regulated terms within biological process (BP) or molecular function (MF) in the plants exposed to HIPVs (red bubbles Fig. 6) and UDVs (blue bubbles, Fig. 6). At 24h after plants were exposed to HIPVs, there was no regulation in the terms related to cellular components (CC) except for the plasma membrane. In contrast, at this time point, HIPV-exposed plants showed that biological processes (BP) related to plant defence, small molecule biosynthetic processes and developmental processes, including anatomical structures, were induced (Fig. 6). The only molecular function (MF) term that was induced at 24 h in HIPV-exposed plants was glucosyltransferase activity (Fig. 6).

KEGG pathways

To elucidate the molecular mechanisms that were modified in HIPV-exposed SS maize plants, DEGs were identified in the two treatments at both 2h and 24 h time points. KEGG pathway enrichment analysis revealed that, in general, metabolic pathways were induced in the HIPV-exposed plants at 24 h after treatment (Fig. 7, Supplementary Table S5). This corroborates with the results of the gene ontology analysis, which showed that for HIPV-exposed plants at 24 h, there was an induction of genes related to small molecule biosynthetic pathways, more specifically, secondary metabolite biosynthesis, such as benzoxazinoid biosynthesis genes *bx8*, amino acid metabolism and carbohydrate metabolism. Priming also seemed to play an important role in genes related to nucleotide metabolism and genetic information processes. Sample analysis at the 2 h time point displayed 39 induced genes in HIPV-exposed plants, while only 9 induced genes were observed in the UDV-exposed plants (Supplementary Table S4). However, these inductions were shown by sample analysis at the 24 h time point to have ceased in HIPV-exposed plants. It is noteworthy that at the 24 h time point, genes for plant hormone signal transduction and lipid metabolism were induced in HIPV-exposed plants compared to UDV-exposed plants, as were genes involved in the metabolism of terpenoids and polyketides (Fig. 7).

Discussion

In this study, the effect of *S. frugiperda* herbivore-induced plant volatiles (HIPVs) emitted by two maize genotypes, Sintetico Spodoptera (SS) and L3, on the defence response of neighbouring maize plants was explored. Volatiles emitted by SS maize plants at 8 h after exposure to HIPVs and addition of *S. frugiperda* larvae (+ Sf) were significantly attractive to the egg parasitoid *T. remus*, whereas volatiles from SS maize plants exposed to UDVs + Sf were not attractive. These data suggest that the SS maize plants were primed for a faster indirect defence response to *S. frugiperda* damage, following pre-exposure to maize HIPVs. Exposure of SS plants to HIPVs + Sf produced an enhanced level of total volatiles at 8-16 h, compared to that produced by other treatments. The non-preference of the egg parasitoid when volatiles from HIPVs + Sf and UDVs + Sf plants were tested simultaneously in Y-tube olfactometer assays suggests that *T. remus* does not distinguish, at the olfactory level, quantitative differences in levels of volatiles emitted by HIPVs + Sf and UDVs + Sf plants. However, this does not mean that primed plants will not enhance biological control in field conditions. The perception of volatiles by natural enemies in field conditions can be affected by different

factors, including the environmental background (Schröder and Hilker 2008), which can enhance, reduce, or completely mask the egg parasitoid response to semiochemicals (Michereff et al. 2016). Therefore, the effect of primed maize plants by HIPVs on biological control should be evaluated under field conditions. In contrast to neighbouring SS plants, L3 plants did not change their chemical profile of volatiles when exposed to HIPVs emitted from L3 plants. *S. frugiperda* damage produces lower levels of HIPVs in L3 compared to other genotypes, including SS (Michereff et al. 2019). When L3 plants were exposed to HIPVs and UDVs emitted from SS genotypes, there was an enhanced volatile production compared to plants that did not receive *S. frugiperda* larvae. This enhanced volatile production was only due to the herbivory of *S. frugiperda* larvae, rather than pre-exposure to HIPVs, and this change did not attract the egg parasitoid. We propose that the levels of HIPVs produced by the L3 genotype are insufficient to attract the egg parasitoids. Quantities of volatile chemical signals emitted by plants are important for plant-to-plant communication (Heil and Ton 2008). Volatile signals at a very low concentration will not be able to induce a complete plant defence response but could instead induce a priming effect. In *Arabidopsis thaliana*, defence priming was induced when the plants were treated with low amounts of β-aminobutyric acid (BABA), and when a high amount of BABA was applied; direct defence occurred **(**van Hulten et al. 2006). SS and L3 plants that were exposed to HIPVs had their volatile production enhanced during the first two hours. This effect could be a directly induced plant defence response or an absorption/re-release of HIPVs. However, for the time periods thereafter, this induction was not observed in the treatments that did not receive biotic stress. In contrast, SS maize plants that received *S. frugiperda* larvae after HIPV exposure, for the time periods thereafter, maintained enhanced production of volatiles and its indirect defence were induced earlier compared to plants that only received the *S. frugiperda* larvae, confirming that the primed response is due a genuine plant-to-plant communication, not absorption and re-release of HIPVs.

According to the statistical analysis, the compounds (*E*)-ocimene, DMNT, (*E*)-β-farnesene, (*Z*)-3-hexenyl acetate, indole, α-humulene and (*E*)-(1*R*,9*S*)-caryophyllene separated the treatments. Work elsewhere showed that maize plants treated with synthetic or naturally-released green leaf volatiles, (*Z*)-3-hexenal, (*Z*)-3-hexen-1-ol and (*Z*)-3-hexenyl acetate were primed for an enhanced defence response when subjected to a second stress, producing higher levels of sesquiterpenes and the phytohormone jasmonic acid (Engelberth et al. 2007). Furthermore, indole was shown to be a key component that induces priming effects on maize and cotton plants **(**Erb et al. 2015, Hu et al., 2018). Hu et al., (2018) reported that maize plants

were primed upon exposure to either indole or indole + (*Z*)-3-hexenyl acetate. A synergistic priming effect was observed for the binary treatment, and the single treatment with (*Z*)-3-hexenyl acetate induced the plant defence. The authors discuss that this response shows that plants have the ability to discriminate different plant volatile blends in the environment. A single green leaf volatile compound cannot be a reliable cue of the presence of the herbivore, since they can be emitted due mechanical damage, for example, or from other organisms, like stink bugs (Blassioli-Moraes et al., 2016). Therefore, information from blends of compounds can be more reliable than a single compound (van Hulten et al. 2006; Hu et al. 2018). In our study, we did not evaluate individual compounds as elicitors. However, indole and green leaf volatiles proposed in those studies (Engelberth et al. 2007, Erb et al. 2015, Hu et al., 2018) were also identified in the HIPV blend of the SS genotype. A previous study reported that maize plants treated with the phytopheromone *cis*-jasmone followed by *Cicadulina storeyi* (Homoptera: Cicadellidae) challenge produced higher levels of three sesquiterpenoids, (*E*)-(1*R*,9*S*)-caryophyllene, (*E*)-bergamotene and (*E*)-β-farnesene (Oluwafemi et al. 2013). Maize plants that were primed by *S. littoralis* HIPVs, followed by wounding and treatment with *S. littoralis* regurgitant, produced higher amounts of (*E*)-β-farnesene, DMNT and indole (Ton et al. 2007). Other species of plants also exhibit enhanced volatile compound induction in primed plants, as observed in the hybrid poplar *Populus deltoides* x nigra (Malpighiales: Salicaceae), which release enhanced levels of DMNT and (*E*)-ocimene after herbivory by *Lymantria dispar* (Lepidoptera: Lymantriidae) (Frost et al. 2008).

The results obtained here showed that the *T. remus* responds to a blend of HIPVs with only three compounds induced i.e. (*E*)-ocimene, DMNT and (*E*)-β-farnesene (HIPVs + Sf 8-16 h). Previously we reported that this parasitoid responded to a blend of maize HIPVs with fifteen compounds induced (Sf 12-24 h) (Michereff et al., 2019). Further studies could evaluate the importance of these components for the foraging behaviour of this egg parasitoid to evaluate the presence of redundant information in the blend of maize HIPVs (Tasin et al. 2007; Bruce and Pickett; 2011, Magalhães et al. 2019). The response to mixtures of HIPVs from the same source, and not only to one specific compound or blend, helps to overcome problems with signalling detectability. A range of studies have shown the importance of these compounds in plant defence, for example DMNT and TMTT have been shown to attract natural enemies (Bruce et al. 2008; Moraes et al. 2009; Tamiru et al. 2011), influence the foraging behaviour of herbivores (Magalhães et al., 2016, Fancelli et al., 2018, Blassioli-Moraes et al., 2019), and elicit overexpression of DMNT biosynthesis genes in *Arabidopsis thaliana* plants,

leading to higher levels of DMNT being emitted and greater plant resistance against *Plutella xylostella* (Chen et al 2021).

Molecular analysis in this study was conducted using HIPV and UDV-exposed SS maize plants that were not subjected to a biotic challenge. RNA-Seq analysis showed that *WRKY TF*, a jasmonate-induced protein, was upregulated in HIPV-exposed plants. The *WRKY* proteins are involved in responses to pathogens and salicylic acid in primed plants (Yamasaki et al. 2005). The jasmonate-induced protein is related to plant defence against chewing insects. This study was able to show that before receiving the second stress treatment, plants exposed to HIPVs undergo important changes in molecular responses; genes such as the *WRKY74*-superfamily *GRMZM2G163418* of TFs and jasmonate-induced protein *GRMZM2G0515*4 are differentially expressed at 2h and 24 h respectively; pathways related to small molecule biosynthesis for plant defence are also upregulated. The *bx*8 gene upregulated at 24 h encodes a glucosyltransferase enzyme that is involved in the accumulation of DIBOA-Glc in plants, (Woüters et al. 2016, Zhou et al. 2018). Changes at the molecular level and in the volatile emission of SS maize plants provide evidence for a priming effect and suggest that this type of induced defence is largely dependent on the quality of the stressor and genotype dependent. Zhang et al (2019) showed that tomato plants exposed to whitefly-induced tomato plant volatiles were more susceptible to whiteflies, because the whitefly-induced tomato plant volatiles prime SA-dependent defences and suppress JA-dependent defences. Jing et al., (2020) showed that DMNT induces plant defence instead of priming. In agreement with our data that not all maize genotypes can be primed by HIPVs, these results showed that not all genotypes are primed by HIPVs and not all HIPVs will have a positive effect on neighbouring plant defences.

Maize plants primed by HIPVs can display greater resistance to herbivory through a stronger and earlier attraction of natural enemies of the herbivore, indicating that specific maize cultivars might be used as sentinel plants, releasing HIPVs to trigger and induce the defence mechanisms of neighbouring plants. Primed plants can “memorize” the information from a previous stress and respond to a similar future stress faster, earlier, stronger or can have their defence triggered by a lower stress level (Hilker et al. 2016). Therefore, these plants may have a selective advantage over plants that are unable to be primed. We are now conducting a study to evaluate the influence of HIPVs on maize direct defence responses, in view of the upregulation of secondary metabolite biosynthesis genes in HIPV-exposed SS maize plants. Furthermore, future field studies could evaluate SS maize plants, which release significant

amounts of HIPVs, for their ability to function as a sentinel plant to prime defence in neighbouring smart plants.

Author contribution

MFFM, MCBM, MAB, JJZ, MB, RAL and PG conceived the ideas and designed methodology; MFFM, PHCS and MCBM collected the data; MFFM, MCBM, RCT, MMCC and PG analysed the data; MFFM, MB, PG and MCBM led writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Conflict of interest

The authors declare that they have no conflict of interest.

Data availability

All data supporting the findings of this study are available within the paper and within its supplementary materials published online.

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Figure captions

Fig. 1 Mean total amount of volatiles produced per hour (ng/h) in phase 3 by Sintético Spodoptera (SS) and L3 maize genotypes after exposure to either HIPVs or UDVs from SS plants and L3 plants in phase 2 and treatment with the fall armyworm (*Spodoptera frugiperda*) larvae in phase 3.

Fig. 1a SS exposed to HIPVs and UDVs from SS plants;

Fig. 1b L3 exposed to HIPVs and UDVs from L3 plants;

Fig. 1c L3 exposed to HIPVs and UDVs from SS plants.

HIPVs + Sf: TP that received SP HIPVs in phase 2 and received larvae of *S. frugiperda*;

HIPVs - Sf: TP that received SP HIPVs in phase 2 and had no further biotic challenge;

UDVs + Sf: TP that received SP UDVs in phase 2 and received larvae of *S. frugiperda*;

UDVs - Sf: TP that received SP UDVs in phase 2 and had no further biotic challenge.

Means with the same letter within a given sampling time range (0-2, 2-4, 4-8, 8-16 or 16-24 h) were not significantly different ($P > 0.05$) by ANODEV and mean comparisons by contrast analyses. NS = non-significant.

Fig. 2 Principal Response Curve (PRC) multivariate analysis of volatiles released by Sintético Spodoptera (SS) and L3 maize genotypes in phase 3 after exposure to either HIPVs or UDVs from SS and L3 plants in phase 2 and treatment with Fall Armyworm, *Spodoptera frugiperda*, larvae in phase 3.

Fig. 2a SS exposed to HIPVs and UDVs from SS plants;

Fig. 2b L3 exposed to HIPVs and UDVs from L3 plants;

Fig. 2c L3 exposed to HIPVs and UDVs from SS plants.

HIPVs + Sf: TP that received SP HIPVs in phase 2 and received larvae of *S. frugiperda*;

HIPVs - Sf: TP that received SP HIPVs in phase 2 and had no further biotic challenge;

UDVs + Sf: TP that received SP UDVs in phase 2 and received larvae of *S. frugiperda*;

UDVs - Sf: TP that received SP UDVs in phase 2 and had no further biotic challenge.

Data represent the response pattern of maize to different treatments with time. The higher absolute value of the variable weight, the more closely the compound response pattern follows the deviation pattern (from the control, UDVs - Sf = 0 line) indicated on the PRC plots.

Fig. 3 Principal component analysis (PCA) ordination for components 1 and 2 of volatile compounds emitted by undamaged maize plants (-Sf), *S. frugiperda* larvae-damaged maize plants (+Sf) and primed plants followed by feeding damage of maize plants (HIPVs + Sf) at the two time ranges evaluated in the behavioral experiments. **Fig. 3a** 4-8 h and **Fig. 3b** 8-16 h. C corresponds to volatile compound: C1 = β-myrcene, C2 = (*Z*)-3-hexenyl acetate, C3 = (*E*)-ocimene, C4 = methyl benzoate, C5 = (*R*,*S*)-linalool, C6 = (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), C7 = indole, C8 = cyclosativene, C9 = (*E*)-(1*R*,9*S*)-caryophyllene, C10 = (*E*)-β-farnesene, C11 = (*E*,*E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT), C12 = (*E*)-2-hexenal, C13 = (*Z*)-3-hexen-1-ol, C14 = camphene, C15 = (*E*)-2-hexenyl acetate, C16 = geranyl acetate, C17 = α-bergamotene, C18 = geranylacetone, C19 = α-humulene and C20 = δ-cadinene.

Fig. 4 First choice response of the egg parasitoid *Telenomus remus* in a Y-tube olfactometer to volatiles of maize (SS genotype) subjected to different treatments. Asterisks indicate significant differences between treatments using the Wald test with χ^2 distribution at the 0.05% significance level. Numbers in parentheses indicate the number of parasitoids that did not respond to any treatment.

HIPVs + Sf: TP that received SP HIPVs in phase 2 and received larvae of *S. frugiperda*;

HIPVs - Sf: TP that received SP HIPVs in phase 2 and had no further biotic challenge;

UDVs + Sf: TP that received SP UDVs in phase 2 and received larvae of *S. frugiperda*;

UDVs - Sf: TP that received SP UDVs in phase 2 and had no further biotic challenge;

+Sf: SP that received larvae of *S. frugiperda*;

-Sf: SP that had no biotic challenge.

Fig. 5 Residence time of the egg parasitoid *Telenomus remus* in a Y-tube olfactometer in response to volatiles from maize (SS genotype) subjected to different treatments. Asterisks indicate significant differences between treatments using the paired *t*-test at the 0.05% significance level.

HIPVs + Sf: TP that received SP HIPVs in phase 2 and received larvae of *S. frugiperda*;

HIPVs - Sf: TP that received SP HIPVs in phase 2 and had no further biotic challenge;

UDVs + Sf: TP that received SP UDVs in phase 2 and received larvae of *S. frugiperda*;

UDVs - Sf: TP that received SP UDVs in phase 2 and had no further biotic challenge;

+Sf: SP that received larvae of *S. frugiperda*;

-Sf: SP that had no biotic challenge.

Fig. 6 Gene ontology (GO) enrichment analysis of UDVs (blue) and HIPVs (red) exposed maize in phase 2 of plant-to-plant communication. Bubble plot shows significant GO terms (FDR<0.05) from differentially expressed genes at 2 h and 24 h after treatment. Dotted lines at X-axis indicate the established cutoff of FDR <0.05. Y-axis label represents GO terms. GO

enrichment factor are represented by bubble sizes. The larger the bubble, the greater the ratio between the frequency observed in the sample and that expected in the genome. Small bubbles mean that the quantity found in the sample is the same (or near the same) as expected in the genome. CC = cellular component, BP = Biological Process and MF = Molecular Function.

Fig. 7 KEGG pathway enrichment analysis for differentially expressed genes (DEGs) in maize following exposure to HIPVs and UDVs. Genes belonging to enriched pathways (Corrected P-value < 0.05) are represented by numbers (left panel) or by its frequency (right panel). At each panel, left and right bars represents genes or its frequency at 2 and 24 h after treatment in phase 2, respectively (see Supplementary Table S4). Red symbolizes plants exposed to HIPVs, and blue symbolizes plants exposed to UDVs. Dark colors represent specific genes; light colors represent genes common to both times after treatment.

Supplementary data

Fig. S1: Protocol used to explore plant-plant communication with Sintético Spodoptera (SS) and L3 maize genotypes. SP = source plants; TP = target plants. Phase 1 = source plant (SP) herbivory or no herbivory; phase 2 = target plant (TP) exposure to SP odour; phase 3 = TP herbivory or no herbivory. Four treatments are generated in phase 3:

HIPVs + Sf: TP that received SP HIPVs in phase 2 and received larvae of *S. frugiperda*;

HIPVs - Sf: TP that received SP HIPVs in phase 2 and had no further biotic challenge;

UDVs + Sf: TP that received SP UDVs in phase 2 and received larvae of *S. frugiperda*;

UDVs - Sf: TP that received SP UDVs in phase 2 and had no further biotic challenge.

Figs. S2, S3 and S4: Volatile compounds of maize genotypes SS and L3 exposed to HIPVs and UDVs of SS and L3 with different treatments.

Fig. S5: Bioassays with parasitoid *Telenomus remus* with L3 maize plants exposed to HIPVs and UDVs of SS genotype.

Fig. S6: Vulcano plots. Figure S5: A) Venn diagram of UDVs and HIPVs exposed plants exclusive (red and blue) and common DEGs (purple). B) Linear correlation (with R 2 values)

analysis of 1,844 UDVs exposed plants DEGs (left panel – red), 1,255 common DEGs (center panel, purple) and1,139 HIPVs exposed plants DEGs (right panel - blue).

Table S1: Statistical values for Fig. 1.

Table S2: Statistical values for Fig. 2.

Table S3: Statistical values for Fig. S2.

Table S4: Differential genes.

Table S5: KEGGS Pathway