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Spodoptera frugiperda CATERPILLARS SUPPRESS HERBIVORE-INDUCED VOLATILE EMISSIONS IN MAIZE

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Abstract – The vast spectrum of inducible plant defenses can have direct negative effects on herbivores, or indirect effects, for instance in the form of herbivore-induced plant volatiles (HIPVs) that attract natural enemies. Various arthropods have evolved ways to suppress plant defenses. To test whether this is the case for caterpillar-induced HIPVs, we compared the volatile induction by *Spodoptera frugiperda* (Lepidoptera: Noctuidae), which is particularly well adapted to feed on maize (*Zea mays* ssp. *mays*), with the induction by three more generalist noctuid larvae. We tested the hypothesis that *S. frugiperda* suppresses HIPV emissions in maize, and thereby reduces attractiveness to natural enemies. HIPV emissions triggered by *S. frugiperda* when feeding on maize were indeed found to be significantly weaker than by *Spodoptera littoralis*, *Spodoptera exigua*, and *Helicoverpa armigera*. The suppression seems specific for maize, as we found no evidence for this when *S. frugiperda* caterpillars fed on cotton (*Gossypium herbaceum*). Artificially damaged maize plants treated with larval regurgitant revealed that HIPV suppression may be related to factors in the caterpillars' oral secretions. We also found evidence that differential physical damage that the caterpillars inflict on maize leaves may play a role. The suppressed induction of HIPVs had no apparent consequences for the attraction of a common parasitoid of *S. frugiperda*, *Cotesia marginiventris* (Hymenoptera: Braconidae). Nevertheless, the ability to manipulate the defenses of its main host plant may have contributed to the success of *S. frugiperda* as a major pest of maize, especially in Africa and Asia, which it has recently invaded.

Key Words – Herbivore-induced plant volatiles, tritrophic interactions, maize, cotton, *Spodoptera exigua*, *Spodoptera frugiperda*, *Spodoptera littoralis*, *Cotesia marginiventris*, parasitoids.

INTRODUCTION

Numerous studies have revealed that plants are equipped with a broad spectrum of defense mechanisms to protect themselves against herbivorous arthropods. Plants can use direct defenses, such as the production of toxic compounds, either constitutively or induced by insect herbivore attack (Howe and Jander, 2008; Karban and Baldwin, 1997; Wu and Baldwin, 2010). In addition, it has been proposed that plants protect themselves indirectly by attracting natural enemies of their herbivores with herbivore-induced plant volatiles (HIPVs) (Dicke et al., 2002; Turlings and Wäckers 2004). The function of HIPVs remains topic of discussion (De Lange et al. 2018; Dicke and Baldwin, 2010; Hare, 2011; Heil 2014; Poelman 2015; Turlings and Erb, 2018), but various studies have shown that they are highly attractive to predators and parasitoids of the herbivores (e.g. De Moraes et al. 1998; Dicke and Sabelis, 1988; Kessler and Baldwin 2001; Thaler 1999; Turlings et al. 1990).

Typically, plants detect elicitors in the oral secretions of arthropods, also known as herbivore-associated molecular patterns, which then triggers the release of volatiles (Acevedo et al. 2015; Erb and Reymond, 2019; Felton and Tumlinson 2008; Schmelz 2015). For example, volicitin present in the regurgitant of *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) larvae induces the emission of HIPVs in maize (*Zea mays* L. ssp. *mays*) (Alborn et al. 1997; Turlings et al. 2000). This and other fatty acid conjugates are also potent elicitors of defense responses in native tobacco (*Nicotiana attenuata*), including the release of volatiles (Halitschke et al., 2003). Similarly, inceptin, isolated from the oral secretions of *Spodoptera frugiperda* Smith, is a potent elicitor of HIPVs in legumes (Carroll et al. 2008; Schmelz et al. 2006). Caeliferins (Alborn et al. 2007) and β-glycosidase (Mattiacci et al. 1995) are further examples of insect-derived elicitors.

90 Not only do arthropods induce plant defenses, they may also produce repressing
91 compounds to suppress or re-direct inducible plant defenses (Alba et al. 2012b; Pieterse and
92 Dicke, 2007; Walling, 2000). In analogy with plant pathogenic microbes, these repressing
93 compounds are commonly referred to as "effectors" (Boller and Sy, 2009; Dangl and Jones,
94 2001; Hogenhout and Bos, 2011). Musser et al. (2002) found that the enzyme glucose oxidase,
95 obtained from oral secretions of the lepidopteran larva *Helicoverpa zea* (Lepidoptera:
96 Noctuidae), is a powerful repressor of toxic nicotine, a direct defense compound of tobacco
97 (*Nicotiana tabacum*), but in tomato (*Solanum lycopersicum*) this enzyme induces defenses
98 (Tian et al. 2012). ATP hydrolyzing enzymes in *H. zea* saliva can suppress direct defenses in
99 tomato (Wu et al. 2012). The regurgitant of Colorado potato beetle, *Leptinotarsa decemlineata*
100 (Coleoptera: Chrysomelidae), suppresses the expression of wound-inducible genes in tomato
101 (Lawrence et al. 2007). Interestingly, orally secreted bacteria are held responsible for this effect,
102 and bacterial flagellin was identified as a key effector protein (Chung et al. 2013). Indeed,
103 microbial endosymbionts or endosymbiont-like pathogens may manipulate plant defenses to
104 benefit their arthropod hosts (Barr et al. 2010; Casteel et al. 2012; Su et al. 2015). In other cases,
105 the compounds responsible for defense repression remain unknown (e.g. Consales et al. 2011).
106 If plants actively recruit the natural enemies of their enemies, it can be expected that
107 specialized herbivores have adapted to circumvent and even suppress such indirect plant
108 defenses, similarly to the suppression of direct defenses (Alba et al. 2012a). Indeed, oral
109 secretions of *H. zea* have been found to suppress the emission of HIPVs in tobacco (Delphia et
110 al. 2006). Furthermore, *S. exigua* oral secretions can decrease transcript levels of regulatory
111 genes involved in volatile terpenoid biosynthesis in barrel clover (*Medicago truncatula*) (Bede
112 et al. 2006). A study by Sarmento et al. (2011) showed that feeding by the spider mite
113 *Tetranychus evansi* suppressed the release of HIPVs from its host plant tomato, although two
114 species of predatory mites (*Phytoseiulus longipes* and *Phytoseiulus macropilis*) were still

115 attracted to the herbivore-infested plants (Sarmento et al. 2011). Therefore, the ecological
116 relevance of manipulation of indirect defenses by herbivores has remained uncertain.

117 In this study, we addressed the possibility that larvae of the moth *S. frugiperda* are
118 capable of suppressing indirect defenses in maize and thereby reduce the plant's attractiveness
119 to their natural enemies. Although *S. frugiperda* is a polyphagous species, it has a strong
120 preference for grasses (Luginbill 1928; Pitre et al. 1983; Sparks 1979), and there are indications
121 that it is adapted to cope with direct defenses specific to grasses, such as silica accumulation
122 (Acevedo 2016). The species tolerates and detoxifies benzoxazinoids, the main direct defense
123 compounds in maize and other grasses (Glauser et al. 2011; Wouters et al., 2014). This further
124 confirms that it is a relative specialist on maize, and, as such, it may also be able to suppress its
125 volatile emissions. In the first study to reveal the potency of caterpillar regurgitants to induce
126 volatile emissions (Turlings et al., 1993), the regurgitant of *S. frugiperda* was indeed one of the
127 least active. Recently, further evidence for the suppressing powers of *S. frugiperda* oral
128 secretions were obtained by Acevedo et al. (2017a, 2018, 2019). In the current study we
129 investigated how this may affect HIPVs and their attractiveness to parasitoids. We compared
130 the volatile blends emitted by maize plants upon feeding by *S. frugiperda* larvae with the blends
131 induced by three generalist lepidopteran larvae, *Spodoptera littoralis* Boisduval, *S. exigua* and
132 *Helicoverpa armigera* Hübner, all of which readily feed on maize in agricultural settings
133 (Luginbill 1928; Hill 1975; Kranz et al. 1977; Sparks 1979; Hill 1987; Fitt 1989).

134 As *S. frugiperda* and *S. exigua* co-occur in Mexico (Blanco et al. 2014), the country of
135 origin of maize (Matsuoka et al. 2002), we looked at differences in damage patterns and volatile
136 emissions between these species in more detail. Also, we compared the volatile blends induced
137 by *S. frugiperda* and *S. exigua* when feeding on cotton (*Gossypium herbaceum* L.), a plant on
138 which *S. frugiperda* can readily feed (Barros et al. 2010; Luginbill 1928; Sparks 1979), but to
139 which it is not specifically adapted. *S. exigua* also readily feeds on cotton (Greenberg et al.

2001). In additional experiments, we compared HIPVs after the application of regurgitant to damaged leaves, using the regurgitant of three different *Spodoptera* species, to test for a possible suppressive effect of *S. frugiperda* regurgitant. In a six-arm olfactometer, we also assessed the attractiveness of plant volatiles induced by *S. frugiperda* and *S. exigua* to the solitary koinobiont endoparasitoid *Cotesia marginiventris* Cresson (Hymenoptera: Braconidae), a very common parasitoid of *S. frugiperda* (Hoballah et al. 2004).

Overall, the results imply that *S. frugiperda* is capable of suppressing induced HIPV emissions in maize, but not in cotton. Although suppression of HIPVs did not result in a reduced attractiveness of maize plants to one of the insect's main, probably well adapted, parasitoids, it is likely to reduce the plant's defenses and in part explain the success of *S. frugiperda* as an important pest of maize.

METHODS AND MATERIALS

Plants. Maize seeds (*Z. mays* ssp. *mays*, variety Delprim) were sown in plastic pots (4 cm diameter, 10 cm high) with fertilized commercial soil (Ricoter Aussaaterde, Aarberg, Switzerland). All plants were kept in a climate chamber (27 ± 2°C; 60% relative humidity; 16 hr light/8hr dark; 50.000 lm/m^2). At the beginning of each experiment, the maize plants were 9-12 days old, had a cotyledon, three fully developed leaves and a fourth one emerging from the whorl. Cotton seeds (*G. herbaceum*) were sown in the same plastic pots and were kept under similar conditions as the maize plants. After three weeks, the cotton plants were transplanted to larger pots. At the beginning of the experiments, the cotton plants were 6-8 weeks old, and had 5 fully developed leaves.

164 *Insects. S. littoralis* eggs were provided by Syngenta (Stein, Switzerland). *S. frugiperda* eggs
165 were provided by Bayer CropScience (Monheim, Germany) or were obtained from an in-house
166 colony (Maag et al. 2014). *S. exigua* eggs were provided by Bayer CropScience or from
167 Entomos (Grossdietwil, Switzerland). *H. armigera* eggs were provided by Bayer CropScience.
168 All insect eggs were incubated at room temperature and larvae were reared on artificial diet
169 until they had reached the second instar. Regurgitant was collected as described by Turlings et
170 al. (1993). *C. marginiventris* wasps were reared as described by Turlings et al. (2004). Initial
171 experiments were performed with all four caterpillar species, while additional experiments
172 focused on the three *Spodoptera* species, or only on *S. frugiperda* and *S. exigua* specifically,
173 the two most representative and co-occurring species. *H. armigera* was not included in further
174 studies because its larvae did not feed well in most of our bioassays, causing notable
175 discrepancies in sample sizes between treatments, which affects the reliability of statistical
176 methods.
177
178 *Detached Leaf Feeding Assays.* For an initial, quick assessment of the feeding habits of the four
179 caterpillar species, we performed detached leaf feeding assays, similar to Rostás and Turlings
180 (2008). A single second-instar larva of each species (n = 8) was weighed and placed in an
181 individual box (2 x 2 cm) with a small piece of maize leaf. After 20 hr of overnight feeding, the
182 leaves were scanned into Adobe Photoshop CS2 version 9.0.2 Consumed leaf area was
183 measured using NIH ImageJ software (http://rsb.info.nih.gov/ij/) as described previously (De
184 Lange et al. 2018). Samples when the larvae did not feed were excluded from the analyses (1
185 sample for *H. armigera*).
186
187 *Measuring Feeding Patterns.* For further comparisons and to allow from more replication we
188 worked only with *S. frugiperda* and *S. exigua*. For a more biologically relevant assessment of

189 the feeding habits of these species, we performed clip-cage assays on whole plants as described
190 by Erb et al. (2011b). A single second-instar larva of either *S. exigua* or *S. frugiperda* (n = 12)
191 was weighed and placed in a small clip-cage (surface 0.8 cm^2) on the youngest full-grown maize
192 leaf. Larvae were allowed to feed for 6 hr and were subsequently weighed again. Larval weight
193 gain was calculated as the final minus the initial weight, and consumed leaf area was determined
194 as described above. When visually characterizing the damaged leaf area, two types of damage
195 could be distinguished: "windowpane" feeding, where the epidermis and mesophyll tissue of
196 only one side of the leaf are ingested, and chewing holes (Erb et al. 2011b; Gouinguené et al.
197 2003). Consumed leaf area was attributed to each type of damage.
198 To determine whether differences in feeding patterns and/or differences in mouth parts
199 explain the observed differences in consumed leaf area between *S. exigua* and *S. frugiperda*,
200 we visually inspected feeding damage as well as larval mouth parts by means of scanning
201 electron microscopy (SEM). Leaf material damaged by both species was fixed in a mix of 2%
202 paraformaldehyde and 2.5% glutaraldehyde in a buffer of 0.1 M sodium cacodylate (pH 7.4).
203 After washing the samples three times in the buffer, they were postfixed in a solution of 1%
204 OsO_4 in buffer for 1 hr, and then washed in the buffer three more times. Larvae of both species
205 were fixed in 70% ethanol. Samples were dehydrated in a graded acetone series, critical-point-
206 dried in CO_2, mounted on stubs, and coated with a thin gold layer by a sputter coater (SCD 005;
207 Bal-Tec, Balzers, Liechtenstein). They were examined at 10kV using a Philips XL-30 scanning
208 electron microscope (FEI/Philips Electron Optics, Hillsboro, OR, USA) as described by
209 Roelfstra et al. (2010) and Kessler et al. (2013).
210
211 *Comparing the Induction of Volatile Emissions by Different Noctuid Caterpillars.* To assess
212 whether feeding by four different caterpillar species induces different HIPV emissions, we
213 conducted a series of volatile collection experiments. Maize plants (n = 12) were placed in a

volatile collection setup under experimental conditions as described previously (De Lange et al. 2016; Ton et al. 2007; Turlings et al. 2004). Infestation by *S. frugiperda*, *S. littoralis*, *S. exigua*, and *H. armigera* was achieved by releasing 4-6, 20-22, 15-16, and 35-37 larvae into the leaf whorl, respectively. The numbers of larvae were chosen to balance the amounts of damage that the larvae inflict (see Results section). After 12-14 hr of feeding, volatiles were collected as described below. The larvae remained on the plants during the volatile collections. Control plants received no larvae. Trials in which one species of larvae fed obviously less than the others were excluded from analysis (8 trials for *H. armigera* and 4 trials for *S. frugiperda*). In several cases, the (*Z*)-3-hexenal peak coeluted with the bacterial volatile 2,3-butanediol (D'Alessandro et al. 2013). Therefore, this compound was not included in the total volatile emission data.

We conducted an additional volatile collection experiment with only *S. frugiperda* and *S. exigua*, two of the most common *Spodoptera* species on maize in the Americas (Blanco et al. 2014; Hernandez-Trejo et al. 2019; O'Day and Steffey 1998; Ortega 1987). This time we used equal numbers of caterpillars for both species. The ten second instar larvae per species were chosen such that the *S. frugiperda* larvae were somewhat smaller, but did equal amounts of damage during the 27 hr feeding period. Larvae were weighed and damage was assessed as described above for the detached leaf feeding assays. Three-hour volatile collections started when the larvae had fed for 6 hr and were repeated when the larvae had fed for 24 hr ($n = 6$).

In a third volatile collection experiment, we compared the induction by *S. frugiperda* and *S. exigua* caterpillars on maize plants and cotton plants. Whereas *S. frugiperda* has been shown to tolerate and detoxify direct defense compounds specific to maize (Glauser et al. 2011; Wouters et al., 2014), there are no indications that it is specifically adapted to feed on cotton. Plants were infested with 4, 8, or 16 larvae of each species into the leaf whorl (maize, $n = 11$-12 for each number of larvae) or onto fully developed leaves (cotton, $n = 6$-7 for each number

239 of larvae). Larvae were left to feed for 16 hr on maize plants, or for 48 hr on cotton plants. The
240 reason for this difference in timing is that in the case of maize the inducible volatiles are emitted
241 within hours after the caterpillars start feeding (Turlings et al. 1998), whereas for cotton it takes
242 at least a day (Loughrin et al. 1994). Control plants received no larvae. After volatile collections,
243 performed as described below, leaves were detached and scanned as described by De Lange et
244 al. (2018), and consumed area was measured for each leaf (cotyledon or leaf 2-4) as described
245 above.
246
247 *Regurgitant Treatments.* To test if the larval oral secretions of the different noctuids play a role
248 in the observed differences in HIPVs, we also conducted experiments with mechanically
249 damaged plants that were treated with different caterpillar regurgitants (De Lange et al. 2016;
250 Erb et al. 2009; Gouinguené et al. 2003; Ton et al. 2007). Maize plants (n = 12-14) were
251 individually placed in the glass volatile collection vessels after two leaves of each maize plant
252 were damaged and treated with regurgitant of *H. armigera*, *S. frugiperda*. *S. littoralis*, or *S.*
253 *exigua*, or wounding only. Wounding was inflicted by punching 26 small holes in two leaves at
254 two different locations with a punching device, to damage a total surface of ~4 cm^2 (4 x ~1
255 cm^2). An amount of 10 µl pure regurgitant of each species was applied on the damaged surface.
256 Wounding and regurgitant treatments took place 12-14 hr before the start of volatile collections
257 and were repeated ~1 hr before the start of volatile collections. Collections were performed as
258 described below.
259 A similar experiment was conducted where we only treated specific leaves (damaged
260 plus regurgitant). This was done to test if differential preferences for leaves among the different
261 species could explain the differences in HIPVs. This was also prompted by a recent paper that
262 showed differences in defensive compounds among leaves of different ages in maize plants
263 with three fully developed leaves (Köhler et al. 2015). Again, after damage and regurgitant

264 treatment, maize plants (n = 4) were placed in the volatile collection vessels. Either the 2nd, 3rd,
265 or 4th leaf of each maize plant was treated with regurgitant of *S. frugiperda*, *S. littoralis*, or *S.*
266 *exigua*, or wounding only. In this case, wounding was inflicted with forceps, to damage a
267 surface of ~2 cm^2 (Erb et al. 2015). An amount of 10 µl pure regurgitant of each species was
268 applied on the damaged surface. Volatile collections started 2 hr after treatment and were
269 repeated 8 hr after treatment.
270
271 *Volatile Collections.* Volatiles were collected as described previously (De Lange et al. 2016;
272 Ton et al. 2007; Turlings et al. 2004) using trapping filters containing 25 mg of 80-100 mesh
273 Super Q adsorbent (Alltech Associates, Inc., Deerfield, IL, USA). For the supplementary
274 collections with smaller *S. frugiperda* and larger *S. exigua* larvae and regurgitant bioassays
275 comparing induction of different leaves we used filters with 25 mg of 80-100 mesh HayeSep Q
276 adsorbent (Ohio Valley Specialty Co., Marietta, OH, USA). Volatile collections lasted 3 hr.
277 Before use, trapping filters were rinsed with 3 ml of dichloromethane; after each collection,
278 they were eluted with 150 µl (Super Q filters) or 100 µl (HayeSep Q filters) of dichloromethane
279 (Suprasolv, GC-grade; Merck, Dietikon, Switzerland). The samples were stored at -80°C before
280 analysis.
281
282 *Analysis of the Volatiles.* Two internal standards (*n*-octane and nonyl acetate, each 200 ng in
283 10 µl dichloromethane; Sigma-Aldrich, Buchs, Switzerland) were added to each sample.
284 Volatiles were analyzed with an Agilent 6850 gas chromatograph equipped with a flame
285 ionization detector (GC-FID). A 3-µl aliquot of each sample was injected in pulsed splitless
286 mode onto an apolar capillary column (HP-1ms, 30 m, 0.25 mm ID, 0.25 µm film thickness;
287 Agilent J&W Scientific, Santa Clara, CA, USA). Helium at constant pressure (18.71 psi) was
288 used as carrier gas. After injection, the temperature was maintained at 40°C for 3 min, then

289 increased to 100°C at 8°C/min and subsequently to 200°C at 5°C/min, followed by a post-run
290 of 3 min at 250°C. The detected volatiles were normalized based on a comparison of their peak
291 areas with those of the internal standards, and identified by comparison of retention times with
292 those from previous analyses (D'Alessandro and Turlings 2005).

293 To confirm the identities of the different peaks, at least one odor sample per larval
294 species was analyzed using a gas chromatograph (Agilent 6890 Series GC System G1530A)
295 coupled to a mass spectrometer (GC-MS; Agilent 5973 Network Mass Selective Detector;
296 transfer line 230°C, source 230°C, ionization potential 70 eV). An aliquot of 2 µl was injected
297 in the pulsed splitless mode onto the same type of column as described above. Helium at
298 constant flow (0.9 ml/min) was used as carrier gas. After injection, the column temperature was
299 maintained at 40°C for 3 min, and then increased to 100°C at 8°C/min and subsequently to
300 220°C at 5°C/min followed by a post-run of 3 min at 250°C. The detected volatiles were
301 identified by comparison of their mass spectra with those of the NIST05 library, by comparison
302 of their spectra and retention times with those of authentic standards, and by comparison of
303 their retention times with those from previous analyses (Loughrin et al. 1994; D'Alessandro and
304 Turlings 2005; Ngumbi et al. 2009). Volatiles that met only one of these criteria were labelled
305 as tentatively identified.
306
307 *Six-arm Olfactometer Bioassays.* To assess a possible effect of the observed differences in
308 HIPV emissions for the attraction of natural enemies, we measured the attractiveness of maize
309 plants induced by *S. exigua* and *S. frugiperda* to one of their principal natural enemies, the
310 parasitoid *C. marginiventris*. Maize plants (n = 14) were placed in glass vessels. Infestation by
311 *S. frugiperda* and *S. exigua* caterpillars was achieved by releasing 4 and 16 larvae into the leaf
312 whorl, respectively, which were left to feed overnight. The numbers of larvae were chosen to
313 balance the amounts of damage that the larvae inflict (see Results section). Control plants

314 received no larvae. Bioassays were performed as described previously (De Lange et al. 2016;
315 Turlings et al. 2004). On randomized positions in every other arm, either a *S. frugiperda*-
316 induced, a *S. exigua*-induced, or a control (non-induced) plant was placed. We used mated naïve
317 two- to four-day-old female *C. marginiventris* wasps (n = 288 wasps with 14 exchanges of odor
318 sources). They were released into the olfactometer in groups of 6 and per day 1-6 groups of
319 wasps were tested. The wasps were given 30 min to make a choice and were thereafter removed
320 in order to release a new group.

321 We performed a similar experiment with cotton plants, to which *S. frugiperda* are not
322 specifically adapted. Bioassays with cotton plants (n = 6) were performed as described above,
323 with a few modifications. Infestation by *S. frugiperda*, and *S. exigua* caterpillars was achieved
324 by releasing 16 larvae of each species onto fully developed leaves, 48 hr before the start of the
325 bioassays. Control plants received no larvae. We used two- to four-day-old naïve mated female
326 *C. marginiventris* wasps (n = 216 wasps with 6 exchanges of odor sources).
327
328 *Statistical Analysis.* For data on larval weight, damage, and volatile emissions, differences
329 between two treatments were analyzed using Student's t-test. Differences between more than
330 two treatments were analyzed using one-way analysis of variance (one-way ANOVA) when
331 data were normally distributed, and Kruskal-Wallis test when data were not normally
332 distributed. All significant effects were subjected to pairwise comparisons using Tukey or
333 Dunn's *post hoc* tests. When necessary, percentage data were arcsine-square root-transformed,
334 and volatile emission data were log-transformed, to improve normality and homogeneity of
335 variance (non-transformed values are reported). Concerning plant volatiles, we analyzed total
336 volatile emissions (i.e., the sum of normalized peak areas for all individual compounds), as well
337 as emissions of individual compounds. For the latter, only herbivore-induced plants were
338 included in the statistical analyses. Correlations between damage and volatile emissions were

analyzed using linear regression, and one-way analysis of covariance (one-way ANCOVA) was conducted to determine differences in the slopes and/or intercepts of the linear regression lines. To compare feeding damage on different maize leaves, and volatile emissions when different maize leaves were damaged, we used two-way ANOVA with treatment and leaf number as factors. Wasp choice data were analyzed using a generalized linear model (GLM) fitted by maximum quasi-likelihood estimation according to Turlings et al. (2004). All analyses were performed with SigmaPlot version 13.0 (Systat Software, San Jose, CA, USA) and the software package R version 3.5.0 (R Core Team 2018).

RESULTS

The Four Caterpillar Species Differ in Leaf Consumption Rate. To compare feeding damage on maize by the four different herbivore species, we assessed the extent of damage after 20 hr of feeding on a detached leaf by single second-instar larvae of each species. All larvae had a similar starting weight (*H. armigera*: 1.69 ± 0.005; *S. littoralis*: 1.68 ± 0.005; *S. exigua*: 1.69 ± 0.002; *S. frugiperda*: 1.68 ± 0.004; weight (mg) ± SE; *Kruskal-Wallis test,* $H = 0.93$, $df = 3$, $P = 0.82$). However, a *S. frugiperda* larva consumed significantly more leaf area than did a single larva of *S. littoralis*, *S. exigua* and *H. armigera* (*one-way ANOVA*, $F_{(3,27)} = 15.56$, $P < 0.001$; Figure 1). Since wounding quantitatively influences HIPV emissions (Gouinguené et al. 2003; Turlings et al. 2004), it was necessary to correct for the observed differences in leaf damage. For this reason, we conducted further experiments with 20-22 *S. littoralis*, 15-17 *S. exigua*, 35-37 *H. armigera*, and 4-6 *S. frugiperda* larvae.

S. frugiperda *Induces the Release of Lower Amounts of Volatiles than* S. exigua*,* S. littoralis*, and* H. armigera*.* All lepidopteran larvae induced a significant amount of volatiles compared to control, non-attacked maize plants, but *S. frugiperda* larvae induced considerably lower

amounts of HIPVs than larvae of the other three species (*one-way ANOVA*, $F_{(4,43)} = 93.05$, $P < 0.001$; Figure 2). Statistical tests for emissions of individual compounds were performed on data for herbivore-induced plants only (not for control plants). *S. frugiperda* feeding triggered lower emissions of the green leafy volatiles (GLVs) (*Z*)-3-hexenyl acetate and (*E*)-2-hexenyl acetate than feeding by *S. littoralis* and *S. exigua*. Most monoterpenes, sesquiterpenes, and esters were also emitted in lower quantities in response to feeding by *S. frugiperda* than in response to feeding by *S. littoralis* and *S. exigua* (Table 1).

An additional volatile collection experiment with only *S. frugiperda* and *S. exigua*, in which we used equal numbers of caterpillars (10 per plant), yielded very similar results. The *S. frugiperda* larvae were smaller at the beginning of the experiment (*S. exigua*: 2.52 ± 0.080; *S. frugiperda*: 1.53 ± 0.048; weight (mg) ± SE; *t-test*, $t = 9.07$, $df = 5$, $P < 0.001$), but since they showed a higher feeding rate, the two species inflicted equal amounts of damage (*S. exigua*: 398.1 ± 59.9; *S. frugiperda*: 336.5 ± 28.4; damage (mm^2) ± SE; *t-test*, $t = 1.26$, $df = 5$, $P = 0.26$). After 6 hr, both larvae induced a significant amount of volatiles compared to control, non-attacked maize plants, but there were no significant differences in total volatile emissions between the two species (*one-way ANOVA*, $F_{(2,15)} = 67.93$, $P < 0.001$; Figure 3a). After 24 hr, total volatile emissions were lower for *S. frugiperda*-damaged plants than for *S. exigua*-damaged plants (*one-way ANOVA*, $F_{(2,15)} = 223.32$, $P < 0.001$; Figure 3b). Again, statistical tests for emissions of individual compounds were performed on data for herbivore-induced plants only (not for control plants). These results show that after 6 hr, several GLVs as well as (*Z*)-β-ocimene, (3*E*)-4,8-dimethyl-1,3,7-nonatriene, and geranyl acetate were released in lower quantities by *S. frugiperda*-damaged plants than by *S. exigua*-damaged plants. After 24 hr, most of the inducible compounds were released in lower quantities by *S. frugiperda*-damaged plants, but not the GLVs (Table 2). These discrepant differences in GLV emissions for the two time points could be due to the initial size differences between the larvae, with the smaller *S.*

389 *frugiperda* causing less physical damage at the beginning of the experiment, resulting in lesser
390 amounts of GLVs being released.
391
392 S. frugiperda *Induces Lower Amounts of HIPVs than* S. exigua *in Maize but not in Cotton.* To
393 examine the relationship between herbivory and HIPV emissions in further detail, we correlated
394 inflicted damage on maize plants with HIPV emissions upon feeding by *S. frugiperda* and *S.*
395 *exigua*. Plant HIPV emissions increased steadily with increasing amounts of consumed leaf area
396 for both *S. exigua* (*linear regression*, $R^2 = 0.48$, $F_{(1,33)} = 30.10$, $P < 0.001$) and *S. frugiperda*
397 (*linear regression*, $R^2 = 0.41$, $F_{(1,34)} = 23.47$, $P < 0.001$). However, the slopes of the regression
398 lines were significantly different (*one-way ANCOVA*, $F_{(1,67)} = 7.80$, $P = 0.007$), confirming that
399 *S. frugiperda* induced lower amounts of HIPVs per unit of leaf damage than *S. exigua* (Figure
400 4a). We also observed that the different lepidopteran species preferred to feed on different
401 maize leaves (*two-way ANOVA*, treatment: $F_{(1,276)} = 0.01$, $P = 0.91$, leaf: $F_{(3,276)} = 29.01$, $P <$
402 0.001, interaction: $F_{(3,276)} = 13.93$, $P < 0.001$) (Supplementary Figure 1). This prompted us to
403 perform an additional experiment, in which we assessed HIPV emissions after treating leaves
404 of different ages (see below).
405 When performing a similar experiment with cotton plants, on which *S. frugiperda* is not
406 specialized, there was also an increase of HIPV emissions with increased damage for both *S.*
407 *exigua* (*linear regression*, $R^2 = 0.37$, $F_{(1,16)} = 9.23$, $P = 0.008$) and *S. frugiperda* (*linear*
408 *regression*, $R^2 = 0.69$, $F_{(1,19)} = 41.35$, $P < 0.001$). For cotton, the slopes of the regression lines
409 did not differ (*one-way ANCOVA*, $F_{(1,35)} = 0.90$, $P = 0.35$), nor did the intercepts (*one-way*
410 *ANCOVA*, $F_{(1,36)} = 0.16$, $P = 0.69$), implying that *S. exigua* and *S. frugiperda* induced similar
411 amounts of HIPVs per unit of leaf damage (Figure 4b). These results provide further evidence
412 that *S. frugiperda* is capable of specifically suppressing HIPV emissions in maize.
413

The Regurgitants of Different Spodoptera *Species Trigger Different Amounts of HIPVs.* Our observation that *S. frugiperda* and *S. exigua* prefer to feed on different maize leaves, prompted us to test if induction of different leaves resulted in the release of different amounts of HIPVs. Therefore, we compared total HIPV emissions after standardized regurgitant treatment of different leaves, using regurgitant from all three *Spodoptera* species. Two hours after treatment, *S. frugiperda* regurgitant resulted in the release of significantly lower total amounts of volatiles than regurgitant of the other species, independent of the leaf that was treated. Overall, *S. exigua* regurgitant induced the highest total quantity of HIPVs, which was significantly higher than in response to wounding only. Treatment with *S. littoralis* regurgitant did not affect HIPV emissions, as it was the same as wounding only, and, interestingly, plants treated with *S. frugiperda* regurgitant released even less HIPVs than the plants with only wounding (*two-way ANOVA*, treatment: $F_{(3,36)} = 45.18$, $P < 0.001$, leaf: $F_{(2,36)} = 0.90$, $P = 0.42$, interaction: $F_{(6,36)} = 0.61$, $P = 0.72$) (Figure 5a). Eight hours after treatment, the leaves that were treated with *S. frugiperda* regurgitant still released considerably less HIPVs than those treated with the regurgitant of the other two *Spodoptera* species. Again, induction with *S. exigua* regurgitant increased HIPV emissions the most and treatment with *S. littoralis* regurgitant was intermediate, but not different from wounding only (*two-way ANOVA*, treatment: $F_{(3,36)} = 13.11$, $P < 0.001$, leaf: $F_{(2,36)} = 0.78$, $P = 0.47$, interaction: $F_{(6,36)} = 1.55$, $P = 0.19$) (Figure 5b). Clearly, these results indicate that the three leaves responded similarly, but that caterpillar regurgitant affected the volatile emissions quite differently. Note that control, non-treated plants were not included in this experiment.

We also conducted an experiment in which we punched 26 tiny holes in two of the leaves and treated the leaves with regurgitant of all four different caterpillar species, 12-14h before HIPV collections. Treatments were repeated ~1h before HIPV collections. In this case, we only found significant differences in volatile emissions between wounding only and

regurgitant of the four species (*one-way ANOVA*, $F_{(4,57)} = 10.57$, $P < 0.001$) (Supplementary Figure 2). The absence of HIPV suppression may be due to the low amount of inflicted damage, or the time points at which HIPV emissions were measured in this experiment.

No Differences between S. frugiperda *and* S. exigua *Feeding at the Microscale.* To study the feeding behavior of *S. frugiperda* and *S. exigua* on maize plants in further detail, we observed the mouth parts of both species as well as leaf tissue damaged by both species under the SEM. At microscale, second-instar *S. frugiperda* (Figure 6a) and *S. exigua* (Figure 6b) larvae looked strikingly similar. For both species, we could observe windowpane feeding, where larvae consume the epidermis and mesophyll from one side of the leaf, while leaving the cuticle and the epidermis of the other side of the leaf intact (Figure 6c,d).

S. frugiperda *Takes Larger Bites than* S. exigua. To further study the feeding damage, we compared larval growth and leaf area eaten on maize plants by *S. frugiperda* and *S. exigua* in a clip-cage. While all larvae had the same starting weight (*S. exigua*: 0.72 ± 0.037; *S. frugiperda*: 0.72 ± 0.032; weight (mg) ± SE; *t-test*, $t = 0.02$, $df = 22$, $P = 0.98$) after feeding for 6 hr, *S. frugiperda* gained significantly more weight than *S. exigua* larvae (*t-test*, $t = 6.46$, $df = 22$, $P < 0.001$; Figure 7a). Furthermore, *S. frugiperda* consumed significantly more leaf area than *S. exigua* (*t-test*, $t = 5.31$, $df = 22$, $P < 0.001$; Figure 7b). When distinguishing two types of damage, *S. frugiperda* chewed relatively more holes, and inflicted relatively less windowpane damage than *S. exigua* (*t-test,* $t = 3.33$, $df = 22$, $P = 0.003$) (Figure 7c,d,e). These results suggest that *S. frugiperda* may have a stealthier way of feeding, avoiding the activation of plant defenses by reducing the number of damaged cells.

No Difference in Wasp Attractiveness of Maize Plants Damaged by S. frugiperda *or* S. exigua. A possible ecological relevance of HIPV suppression by *S. frugiperda* was studied by comparing attraction of *C. marginiventris* parasitoids to HIPVs induced by similar amounts of leaf damage incurred by *S. exigua* and *S. frugiperda* larvae. The wasps strongly preferred the odor of herbivore-induced maize plants over the odor of non-induced plants (control) and empty arms, but did not show a preference for either *S. exigua*- or *S. frugiperda*-attacked plants (*GLM*, $F_{(3,284)} = 22.20$, $P < 0.001$; Figure 8a). These results imply that the attraction of *C. marginiventris*, a very common parasitoid of *S. frugiperda*, is not affected by *S. frugiperda*'s capacity to suppress maize HIPV emissions.

No Difference in Wasp Attractiveness of Cotton Plants Damaged by S. frugiperda *or* S. exigua. We also compared the attractiveness of cotton HIPVs to *C. marginiventris* parasitoids between plants that were damaged by *S. exigua* or *S. frugiperda* larvae. Again, the wasps preferred the odor of herbivore-induced plants over non-induced plants (control) and empty arms, but showed no significant difference in their choices for *S. exigua*- and *S. frugiperda*-damaged plants (*GLM*, $F_{(3,212)} = 19.93$, $P < 0.001$; Figure 8b).

DISCUSSION

This study confirms that *S. frugiperda* larvae are capable of specifically suppressing herbivore-induced volatiles in maize. This suppression is associated with lower elicitation activity of the regurgitant and differences in leaf damage patterns. The plant's attractiveness to a common parasitoid wasp does not seem to be affected by this HIPV suppression, however, suggesting that parasitoids can overcome plant defense manipulation by *S. frugiperda*.

The exact mechanism behind the observed suppression remains to be elucidated, but we provide evidence that it involves compounds present in the insect's regurgitant (Figure 5). Sarmento et al. (2011) found something similar for the spider mite *T. evansi*, which suppresses HIPV emissions in tomato compared to *T. urticae* Koch, yet the predatory mite *P. longipes* did not distinguish between plants induced by either spider mite species. Effector-like proteins in the saliva of both spider mite species were shown to suppress defenses when expressed in *Nicotiana benthamiana* (Villarroel et al. 2016). Putative defense suppression activity has also been reported for the regurgitant of *S. exigua* and *S. frugiperda*, as the regurgitants of both species have been shown to suppress GLV emissions in ground maize tissue (Jones et al. 2019). *S. exigua* regurgitant reportedly decreased transcript levels of terpene-related genes in *M. truncatula* (Bede et al. 2006). It has also been shown that *S. frugiperda* regurgitant contains bacteria that can downregulate the activity of two defensive proteins in tomato (Acevedo et al. 2017a). *S. frugiperda*, *S. exigua*, and *S. littoralis* regurgitant all contain volicitin, which induces HIPV emissions in maize (Alborn et al. 1997; Spiteller et al. 2001; Turlings et al. 2000). It is possible that the levels of volicitin and volicitin-related compounds in the regurgitant of the three species is different, as has been reported for other lepidopteran species (Mori et al. 2003). Volicitin does not induce HIPV release in lima bean (*Phaseolus lunatus*), cotton (*Gossypium hirsutum*), or cowpea (*Vigna unguiculata*) (Schmelz et al. 2009; Spiteller et al. 2001), indicating that the effects of elicitors, and possibly also suppressors, is host plant-specific (Louis et al. 2013). Our results imply that, in addition to elicitors, *S. frugiperda* regurgitant contains effectors that are specifically active in maize. Alternatively, *S. frugiperda* regurgitant may contain lower levels of elicitors than the regurgitant of the other tested lepidopteran species.

A recent study showed that protein content in *S. frugiperda* regurgitant differs depending on insect diet (Acevedo et al. 2017b). In fact, two *S. frugiperda* strains occur, a "corn strain" associated with maize and cotton (*Gossypium* spp.), and a "rice strain" associated with

512 rice (*Oryza sativa*). Individuals of both strains displayed differential gene expression when fed
513 on the same diet, indicating alimentary divergence and possible specialization (Roy et al. 2016).
514 Regurgitant of the corn strain suppresses the activity of a defensive protein in Bermuda grass
515 (*Cynodon dactylon*), but not in maize, whereas the regurgitant of the rice strain induces the
516 activity of defensive proteins in both plants. Larvae seem to benefit from plant defense
517 suppression, as lower levels of defensive protein activity were correlated with higher weight
518 gain. Interestingly, the authors propose that changes in larval saliva content could lead to
519 adaptation to novel food sources (Acevedo et al. 2018). Suppressing factors in *S. frugiperda*
520 regurgitant may contribute to its status as a major pest in maize, and its rapid invasion in Africa
521 and Asia, which is currently taking place (Day et al. 2017; Stokstad 2017; Nagoshi et al. 2019).
522 Our experiments focused on HIPV emissions, and revealed that *S. exigua* regurgitant
523 strongly induces HIPVs, while *S. frugiperda* regurgitant represses the emissions (Figure 5). The
524 relatively low HIPV amounts emitted by maize plants treated with *S. frugiperda* regurgitant is
525 in line with the findings by Turlings et al. (1993). When they incubated excised maize seedlings
526 in diluted regurgitant of different lepidopteran species, the regurgitant of *S. frugiperda* was one
527 of the least active. Another, more recent, study showed that *S. frugiperda* regurgitant induces
528 the release of HIPVs in maize, but there were significant differences between the two maize
529 varieties that were tested (Block et al. 2018). A possible explanation for the discrepancies
530 between the studies is that different maize varieties were used, and it is known that there is a
531 high level of variability in defense responses in different plant genotypes (Degen et al. 2004;
532 De Lange et al. 2019; Erb et al. 2011a). Schmelz et al. (2009) found that the elicitor volicitin
533 does not induce volatiles in all maize varieties, indicating that the effects of elicitors, and
534 possibly also suppressors, may be genotype specific. The type of wounding and exposure to
535 regurgitant may also make a difference. When we used a different method to wound the plants,
536 and volatiles were collected 12-14 hr after treatment (which was repeated 1 hr before

collections), rather than after 2 and 8 hr, the application of *S. frugiperda*, *S. littoralis*, *S. exigua*, and *H. armigera* regurgitant induced very similar amounts of HIPVs in maize plants, and the emissions were significantly higher than for wounding alone (Supplementary Figure 2). It is, therefore, possible that defense suppression properties of the regurgitant change with time. Alternatively, defense suppression may result from interactions between wound-derived and herbivore-derived molecular patterns, resulting in different outcomes depending on the method used for wounding and application of oral secretions. Future studies on the oral secretions of *S. frugiperda* larvae should determine if possible effectors from their saliva (Musser et al. 2006) or other compounds in their regurgitant are responsible for the suppression of maize HIPVs. Future studies should also include other plant species, to reveal whether *S. frugiperda*'s suppressive ability is truly limited to maize.

Besides differences in herbivore-derived elicitors, it could also be that the observed variations in HIPV quantities are due to distinct feeding behaviors that lead to differences in the type of damage caused by the lepidopteran species. Two experiments showed that *S. frugiperda* reduced emissions of monoterpenes, homoterpenes, sesquiterpenes, aromatics, and esters, compared to *S. exigua* feeding, but there were no consistent reductions in emissions of GLVs (Tables 1, 2), except in the early collection (after 6 hr) of the second experiment, when the smaller *S. frugiperda* probably had inflicted less damage than the *S. exigua* larvae. That GLVs can be subject to manipulation by insects was shown by Allman and colleagues, who found isomeric rearrangement of GLVs by caterpillars (Allman and Baldwin, 2010; Allman et al., 2013). Moreover, Jones et al. (2019) found that caterpillar regurgitant, including that of *S. frugiperda* and *S. exigua*, can suppress the emission of GLVs in ground maize tissue. These studies suggest that GLVs are particularly important for plant defense and that it is worthwhile to further explore how and why caterpillars have evolved to reduce their emissions (Jones et al., 2019). In our case, evidence for GLV manipulation remains inconclusive.

562 The fact that *S. frugiperda*-infested and *S. exigua*-infested maize plants were equally
563 attractive to *C. marginiventris* wasps suggests that, at least in the case of this parasitoid that
564 frequently parasitizes *S. frugiperda*, its larvae do not benefit from their ability to suppress HIPV
565 induction (Figure 8a). *S. frugiperda*-infested and *S. exigua*-infested cotton plants were also
566 equally attractive to the parasitoid (Figure 8b). *C. marginiventris* is a generalist that attacks a
567 wide variety of early instar lepidopteran larvae (Bahena-Juárez 2008; Cave 1995) and is a very
568 common natural enemy of *S. frugiperda* (Cortez-Mondaca et al. 2012; De Lange et al. 2014;
569 Hoballah et al. 2004; Jourdie et al. 2008; Molina-Ochoa et al. 2004; Von Mérey et al. 2012), as
570 well as *S. exigua* (Alvarado-Rodriguez 1987; Stewart et al. 2001). Therefore, it is to be expected
571 that the wasp has evolved to readily recognize plant volatiles induced by suitable hosts. Indeed,
572 *C. marginiventris* is attracted to herbivore-induced volatiles of maize, teosintes (i.e., the wild
573 ancestors of maize), cotton (*G. hirsutum*) and cowpea (De Lange et al. 2016; Tamò et al. 2006)
574 and shows strong antennal responses to volatiles from these plants (Gouinguené et al. 2005;
575 Ngumbi et al. 2009). From several laboratory studies we already knew that total quantities of
576 HIPVs are not of key importance for the attraction of *C. marginiventris* (Block et al. 2018;
577 D'Alessandro and Turlings 2005; Fritzsche Hoballah et al. 2002; Sobhy et al. 2012). This is
578 again shown here, and our results also support the notion that minor, as yet unknown
579 compounds in the HIPV blends may be essential for the attraction of *C. marginiventris*
580 (D'Alessandro et al. 2009). *S. frugiperda* and *S. exigua* are attacked by numerous natural
581 enemies in their natural habitat (Cortez-Mondaca et al. 2012; Stewart et al. 2001; Von Mérey
582 et al. 2012), and it can be expected that other parasitoids or predators are affected by changes
583 in the maize HIPV blend. Hence, the full ecological implications for HIPV suppression on
584 interactions with the third trophic level remain to be determined.

585 We found that *S. frugiperda* and *S. exigua* had distinct preferences for specific leaves to
586 feed on. This finding was corroborated by Köhler et al. (2014). Using maize plants with three

587 up to seven leaves, they found that *S. frugiperda* prefers younger leaves while *S. littoralis*
588 prefers older leaves; the younger leaves were associated with higher levels of direct defense
589 compounds, which *S. frugiperda* can tolerate (Glauser et al. 2011). We found a similar
590 difference in leaf preference using younger maize plants with four leaves (Supplementary
591 Figure 1), but this apparently does not explain the difference in HIPV emissions. Induction of
592 the different leaves resulted in very similar amounts of volatiles (Figure 5).

593 An increasing number of studies have shown that arthropod pests can manipulate plant
594 defenses, from insect eggs with defense-suppressing effects (Bruessow et al. 2010; Peñaflor et
595 al. 2011) to whiteflies (Kempema et al. 2007; Zarate et al. 2007), aphids (Elzinga et al. 2014;
596 Naessens et al. 2015), spider mites (Sarmento et al. 2011; Schimmel et al. 2017), and beetles
597 (Lawrence et al. 2007). Specific feeding patterns (Dussourd 2017), as well as suppressing
598 proteins (Elzinga et al. 2014; Naessens et al. 2015; Villarroel et al. 2016) and bacteria (Chung
599 et al. 2013; Acevedo et al. 2017) in arthropod oral secretions are responsible for the suppression.
600 A recent study showed that even compounds in *S. frugiperda* frass can suppress defenses in
601 maize (Ray et al. 2016). Hence, defense manipulation appears to be quite common.

602 In summary, we show here that larvae of *S. frugiperda*, a ferocious pest that is
603 particularly well adapted to feed on maize, is able to repress HIPV emissions in maize.
604 However, the reduced emissions did not change the attractiveness of infested plants to a
605 common and important natural enemy. *S. frugiperda* recently appeared in Africa and Asia,
606 where it is rapidly spreading and causing tremendous crop losses. Sustainable control options
607 are badly needed. Unraveling the mechanisms employed by the pest to manipulate their host
608 plants will provide a better understanding of its adaptations to maize and will set the stage for
609 the development of novel crop protection strategies that could interfere with its ability to
610 overcome and manipulate maize defenses.

611

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961

962 **Figure legends**

963 **Fig. 1** Herbivory of different lepidopteran larvae on detached maize leaves. Values represent
964 average amounts of leaf area consumption (± SE) (*n* = 7-8). Species: *Helicoverpa armigera*
965 (*H.a.*), *Spodoptera littoralis* (*S.l.*), *Spodoptera exigua* (*S.e.*), and *Spodoptera frugiperda* (*S.f.*).
966 Different letters indicate significant differences (*one-way ANOVA*, $P < 0.05$)

967

968 **Fig. 2** Volatile emissions of maize plants infested with different lepidopteran larvae. Values
969 represent average total amounts of volatiles (± SE), i.e. the sum of normalized peak areas for
970 all individual compounds (*n* = 4-12). Treatments: Control (C), feeding by *Helicoverpa*
971 *armigera* (*H.a.*), *Spodoptera littoralis* (*S.l.*), *Spodoptera exigua* (*S.e.*), or *Spodoptera*
972 *frugiperda* (*S.f.*). Volatiles were collected after 12-14h of feeding. Because of coelution with
973 another compound, (*Z*)-3-hexenal was not included in the total volatile emission data. Different
974 letters indicate significant differences (*one-way ANOVA*, $P < 0.05$)

975

976 **Fig. 3** Volatile emissions of maize plants 6h (a) and 24h (b) after infestation with lepidopteran
977 larvae. Values represent the average total amounts of volatiles (± SE), i.e. the sum of normalized
978 peak areas for all individual compounds (*n* = 6). Treatments: feeding by *Spodoptera exigua*
979 (*S.e.*) or *Spodoptera frugiperda* (*S.f.*). At the start of the experiment, *S. frugiperda* larvae were
980 smaller than *S. exigua* larvae, so that the two species inflicted equal amounts of damage.
981 Different letters indicate significant differences (*t-test*, $P < 0.05$). ns = not significant

982

983 **Fig. 4** Correlation between herbivore-inflicted damage and total volatile emissions in maize (a)
984 and cotton (b). Open diamonds represent *Spodoptera exigua* and filled triangles represent
985 *Spodoptera frugiperda*. The dashed line represents the linear regression line for *S. exigua*
986 (maize: $R^2 = 0.48$; cotton: $R^2 = 0.37$) and the solid line represents the linear regression line for

987 *S. frugiperda* (maize: $R^2 = 0.41$; cotton: $R^2 = 0.69$). For maize, $n = 35$-36 and for cotton, $n = 18$-
988 21. For both *S. frugiperda* and *S. exigua*, on both maize and cotton, there was a positive linear
989 relationship between amount of damage and volatile emissions (*linear regression*, $P < 0.005$).
990 An asterisk indicates significant differences between the slopes of the linear regression lines
991 (*one-way ANCOVA*, $P < 0.05$)
992
993 **Fig. 5** Volatile emissions of maize plants 2h (a) and 8h (b) after different leaves were treated
994 with larval regurgitant. Values represent the average total amounts of volatiles (± SE), i.e. the
995 sum of normalized peak areas for all individual compounds ($n = 4$). Treatments: Wounding
996 only (W), regurgitant application of *Spodoptera littoralis* (*S.l.*), *Spodoptera exigua* (*S.e.*), or
997 *Spodoptera frugiperda* (*S.f.*). Wounding was inflicted with forceps. Different letters indicate
998 significant differences between regurgitant treatments, represented by the line above the bars
999 (*two-way ANOVA*, $P < 0.05$). There were no significant differences between the different leaves
1000 (*two-way ANOVA*, $P > 0.05$)
1001
1002 **Fig. 6** Scanning electron microscopy (SEM) images of *Spodoptera* larvae and the damage they
1003 inflict on maize plants. (a) Second-instar *Spodoptera frugiperda* larva. (b) Second-instar
1004 *Spodoptera exigua* larva. (c) Damage inflicted by *S. frugiperda*. (d) Damage inflicted by *S.*
1005 *exigua.* Black arrows indicate undamaged leaf tissue, while white arrows indicate damaged leaf
1006 tissue. The larvae inflict so-called windowpane damage, consuming the epidermis and
1007 mesophyll from one side of the leaf, while leaving the cuticle and the epidermis of the other
1008 side of the leaf intact
1009
1010 **Fig. 7** Weight gain and feeding damage of *Spodoptera frugiperda* and *Spodoptera exigua* larvae
1011 on maize plants. (a) Absolute weight gain (± SE) of the larvae after feeding for 6 hr in a small

clip-cage. (b) Total amount of damage (± SE) inflicted by the larvae. (c) Different types of feeding damage (± SE). For all measurements, n = 12. (d) A representative example of feeding damage of *S. exigua*. (e) A representative example of feeding damage of *S. frugiperda*. Two types of feeding damage were distinguished: grey bars and arrows indicate windowpane feeding while white bars and arrows indicate chewing holes. An asterisk indicates significant differences (*t-test*, $P < 0.05$)

Fig. 8 Responsiveness of naïve female *Cotesia marginiventris* parasitoid wasps to volatiles of *Spodoptera exigua* (*S.e.*)- and *Spodoptera frugiperda* (*S.f.*)-induced maize (a) and cotton (b) plants in a six-arm olfactometer. Values represent the average number of wasps per release of 6 wasps (± SE). Control: non-induced plants. Empty: empty vessels (average value of three vessels). The pie chart indicates the proportion of wasps choosing an arm. For (a), n = 288 wasps with 14 exchanges of odor sources. For (b), n = 216 wasps with 6 exchanges of odor sources. Different letters indicate significant differences (*GLM*, $P < 0.05$)

Tables

Table 1 Individual volatiles emitted by herbivore-induced maize plants

Table 2 Individual volatiles emitted by maize plants, 6h and 24h after herbivore induction

Supplementary material

Supplementary Fig. 1 Herbivore-inflicted damage on different maize leaves. Values represent average percentage of leaf area consumption out of total consumption (± SE) (n = 35-36). Leaf 1 represents the cotyledon. Treatments: feeding by larvae of *Spodoptera exigua* (*S.e.*) or *Spodoptera frugiperda* (*S.f.*). Different letters indicate significant differences between leaves within each species, while asterisks indicate significant differences between species for individual leaves (*two-way ANOVA*, $P < 0.05$). These results correspond to the data in Fig. 3

Supplementary Fig. 2 Volatile emissions of maize plants treated with larval regurgitant. Values represent the average total amounts of volatiles (± SE), i.e. the sum of normalized peak areas for all individual compounds (n = 12-14). Treatments: Wounding only (W), regurgitant application of *Helicoverpa armigera* (*H.a.*), *Spodoptera littoralis* (*S.l.*), *Spodoptera exigua* (*S.e.*), or *Spodoptera frugiperda* (*S.f.*). Wounding was inflicted with a punching device, and volatiles were collected 12-14 hr after treatment (which was repeated 1 hr before collections). Different letters indicate significant differences (*one-way ANOVA*, $P < 0.05$)