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Dispensing synthetic green leaf volatiles in maize fields increases the release of sesquiterpenes by the plants, but has little effect on the attraction of pest and beneficial insects

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ABSTRACT

Maize plants respond to feeding by arthropod herbivores by producing a number of secondary plant compounds, including volatile organic compounds (VOCs). These herbivore-induced VOCs are not only known to attract natural enemies of the herbivores, but they may also prime inducible defences in neighbouring plants, resulting in stronger and faster defence responses in these VOC-exposed plants. Among the compounds that cause this priming effect, green leaf volatiles (GLVs) have received particular attention, as they are ubiquitous and rapidly emitted upon damage. In this study, we investigated their effects under realistic conditions by applying specially devised dispensers to release four synthetic GLVs at physiologically relevant concentrations in a series of experiments in maize fields. We compared the VOC emission of GLV-exposed maize plants to non-exposed plants and monitored the attraction of herbivores and predators, as well as parasitism of the caterpillar *Spodoptera frugiperda*, the most common herbivore in the experimental maize fields. We found that maize plants that were exposed to GLVs emitted increased quantities of sesquiterpenes compared to non-exposed plants. In several replicates, herbivorous insects, such as adult *Diabrotica* beetles and *S. frugiperda* larvae, were observed more frequently in GLV-treated plots and caused more damage to GLV-exposed plants than to non-exposed plants. Parasitism of *S. frugiperda* was only weakly affected by GLVs and overall parasitism rates of *S. frugiperda* were similar in GLV-exposed and non-exposed plots. The effects on insect presence depended on the distance from the GLV-dispensers at which the plants were located. The results are discussed in the context of strategies to improve biological control by enhancing plant-mediated attraction of natural enemies.

Keywords:
Green leaf volatiles, Maize, Priming, Herbivore-induced plant volatiles, Parasitoids, *Spodoptera frugiperda*

1. Introduction

Plants that are attacked by insect herbivores emit volatile organic compounds (VOCs) that attract predatory and parasitic insects (Arimura et al., 2009; Heil, 2008; Turlings and Wäckers, 2004; Dicke et al., 2003; Baldwin, 2010) and even birds (Mäntylä et al., 2008). Besides their role in plant–animal interactions, VOCs have also been implicated in plant–plant interactions (Arimura et al., 2001; Baldwin, 2010). For instance, when plants are exposed to herbivore-induced VOCs from other plants their defence mechanisms are triggered faster and with greater magnitude upon subsequent herbivore attack (Engelberth et al., 2004; Heil and Kost, 2006; Kessler et al., 2006). In laboratory experiments with maize seedlings, this so-called "priming" effect has also been shown to initially enhance the emission of VOCs upon feeding damage by larvae of *Spodoptera littoralis*, resulting in a stronger attraction of *Cotesia marginiventris*, an important parasitoid of *Spodoptera* larvae (Ton et al., 2007). VOC emissions of neighbouring plants can also directly affect herbivore resistance of plants at the metabolic level (Broz et al., 2010). Moreover, adsorbed VOCs can be re-released from the plants (Choh et al., 2004; Karban, 2010; Himanen et al., 2010). The VOC blend emitted by maize plants upon herbivore damage consists of a plethora of compounds of different chemical groups, such as green leaf volatiles (GLVs), alcohols, aromatics, mono-, homo- and sesquiterpenes (D'Alessandro and Turlings, 2006; Paré and Tumlinson, 1999; Turlings et al., 1990).

Although it is not yet known which are the key compounds for parasitoid attraction, recent findings show that not all VOCs play an equally important role in the attraction of parasitoids (D'Alessandro and Turlings, 2005; D'Alessandro et al., 2009; Snoeren et al., 2010). Masking compounds that repel parasitoids or that do not allow them to perceive the attractive compounds have been identified in maize (D'Alessandro and Turlings, 2005), as well as in other systems (Takabayashi et al., 1994; Snoeren et al., 2010).

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Moreover, some compounds are only attractive if they are presented in the right context, i.e. together with the right background odours (Mumm and Hilker, 2005).

A group of compounds that have received increased attention in plant–insect interactions over the last few years are the GLVs, C6-alcohols, aldehydes and their esters that derive from the lipoxygenase pathway (LOX) (Bruinsma et al., 2010). These compounds are emitted immediately upon herbivore damage (Turlings et al., 1998b). The metabolic pathways and synthesis have been unravelled for several compounds including (*Z*)-3-hexenyl-acetate (D'Auria et al., 2007; Arimura et al., 2008), (*Z*)-3-hexenol (Farag et al., 2005) and several studies suggest that GLVs play a key role not only in insect attraction (Allmann and Baldwin, 2010; Halitschke et al., 2008; Shiojiri et al., 2006; Unsicker et al., 2009; Whitman and Eller, 1990), but also in priming of herbivore-induced defences (Engelberth et al., 2004; Frost et al., 2008; Heil and Kost, 2006; Ton et al., 2007), activation of pathogen defence (Yi et al., 2009), or direct induction of defence mechanisms against herbivores (Engelberth et al., 2007; Farag et al., 2005; James and Grasswitz, 2005; Ruther and Kleier, 2005; Walling, 2000; Zeringue, 1992).

The exact mechanism behind GLV-mediated plant signalling is as yet unknown, however, it is evident that small quantities can already trigger priming in plants (Heil and Ton, 2008; Heil and Walters, 2009; Farag and Pare, 2002). Moreover, several studies show that GLVs are attractive to parasitoids (Degenhardt, 2009; Hoballah and Turlings, 2005; Whitman and Eller, 1990; Bruce et al., 2010) and predators (Allmann and Baldwin, 2010). In the field, the attraction of insect predators and parasitoids to synthetic GLVs has been confirmed with the use of trapping and monitoring methods (James, 2003a,b). However, certain herbivores, in particular Coleoptera (Halitschke et al., 2008; Hansson et al., 1999; Larsson et al., 2001; Bruce et al., 2005) are also known to be attracted to GLVs. Therefore there may be a cost to the release of GLVs (Heil and Walters, 2009) and it is not clear whether the application of GLVs in the field would result in an overall reduction in herbivore damage. In order to understand to what extent GLVs can play a role in biological control it is not sufficient to just measure the attraction of beneficial insects, the effect of GLVs on defence responses in GLV-exposed plants and on herbivores should also be taken into account.

In this study we investigated the effects of four synthetic GLVs (*Z*)-3-hexenal, (*Z*)-3-hexenol, (*E*)-2-hexenal, and (*Z*)-3-hexenyl acetate, released at physiological relevant concentrations from specifically developed dispensers on the VOC emission of GLV-exposed plants in the laboratory and in a maize field in Mexico. We then measured herbivory, parasitism and predation of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), to better understand whether the application of synthetic GLVs could be used to enhance biological control of this important lepidopteran pest on maize in the New World.

2. Results

2.1. VOC emission by GLV-exposed plants in the laboratory

In general, maize plants that were exposed to GLVs in the laboratory (Fig. 1) for 16 h emitted larger amounts of VOCs than maize plants not exposed to GLVs (Fig. 3) and the emissions differed between the different sampling periods after induction. At sampling period 0.0–2.5 h the main VOCs found were GLVs. However these were not taken into account, as we could not distinguish between the GLVs emitted by the dispenser from the GLVs emitted by the maize plant. At this time-point, the other VOCs were emitted in slightly higher quantities by GLV-exposed plants, but without statistical difference. At time point 2.5–5.0 h, GLV-exposed maize plants emitted slightly larger amounts of sesquiterpenes. At time-point 5.0–7.5 h, sesquiterpenes were emitted at significantly higher levels ($F_{1,21} = 4.34$, $P < 0.049$), particularly (*E*)-β-farnesene ($F_{1,21} = 4.88$, $P < 0.039$). Small amounts of induced volatiles were still emitted at 24.0–26.5 h after mechanical induction and (*E*)-α-bergamotene was emitted in slightly larger amounts by GLV-exposed plants ($F_{1,21} = 3.04$, $P < 0.096$).

2.2. VOC emission by GLV-exposed plants in the field

In the field, sesquiterpenes were emitted in significantly larger amounts by plants that were exposed to GLVs (152.41 ± 94.08 ng/3 h), compared to non-exposed control plants (26.62 ± 8.36

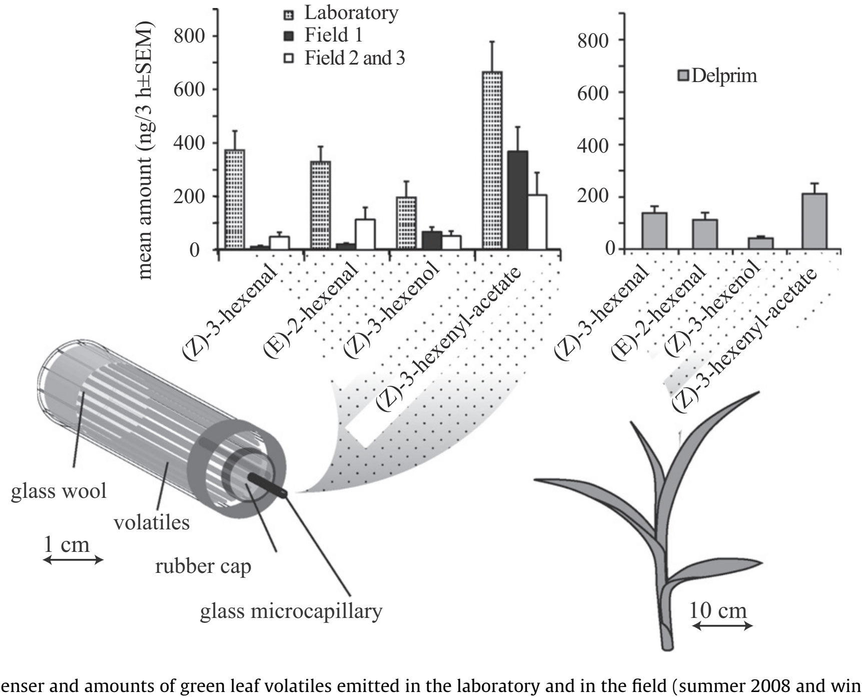

Fig. 1. Schematic of the VOC dispenser and amounts of green leaf volatiles emitted in the laboratory and in the field (summer 2008 and winter 2009). Delprim maize plants emitted comparable amounts.

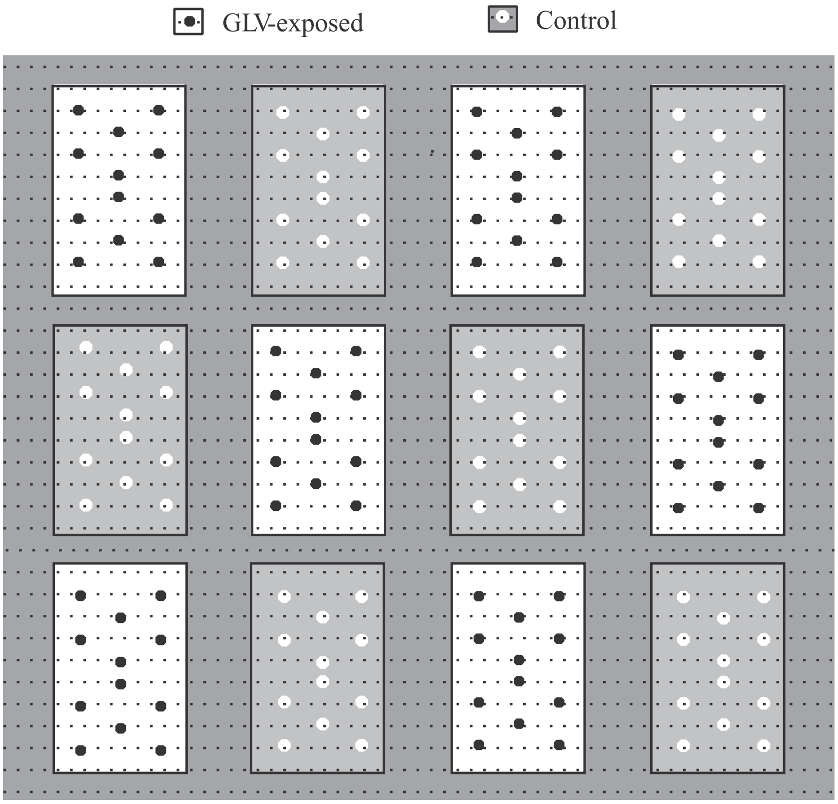

Fig. 2. Design of a field experiment. The field was divided in 4 m × 10 rows-sized plots. White squares were plots with plants exposed to GLVs. Grey squares were plots with control plants. Big black dots represent plants directly exposed to GLV dispensers. Big white dots represent plants exposed to empty control dispensers. Small black dots represent plants that were not exposed directly to dispensers. The distance between rows was 70 cm. Length of rows with in each square (plot) was 4 m. Plants outside the squares served as a buffer between plots and were not considered in the experiment. Three such field experiments were conducted at different time points.

ng/3 h) (Wilcoxontest: $P < 0.047$) (Fig. 2). The sesquiterpene blend included cycloisosativene, alpha-copaene, (*E*)-β-caryophyllene, (*E*)-β-farnesene, (*E*)-α-bergamotene, as well as several non-identified sesquiterpenes. The emission of aromatic compounds (methyl salicylate, phenethyl acetate and non-identified aromatics) ($P < 0.194$), the homoterpene (*E*)-4,8-dimethyl-1,3,7-nonatriene ($P < 0.667$), and monoterpenes (β-myrcene, trans-ocimene, and linalool) ($P < 0.313$) did not differ between GLV-exposed and control plants (Fig. 5).

2.3. Herbivore damage

Fig. 4 illustrates the damage caused by *S. frugiperda* and *Diabrotica*, the two most abundant herbivores in the experimental maize fields. In general there was a tendency of higher damage by *S. frugiperda* in GLV-exposed plants compared to control plants. In particular, in field 2 this tendency towards a higher damage by *S. frugiperda* in GLV-exposed plants on the eighth day was almost significant ($F_{1,430} = 2.802$, $P < 0.095$) (Fig. 4B). Interestingly, compared to the plant at <0.1 m from the GLV-dispenser, there was a significant difference between distances from the dispenser, (0.1–0.3 m: $t = -2.132$, $P < 0.033$ and at 0.3–1.0 m: $t = -1.920$, $P < 0.055$), but there was no overall difference between GLV-exposed and control plants ($t = 1.10$, $P < 0.2715$). However, there were significant field ($t = -9.744$, $P < 0.001$) and sampling day effects ($t = 9.188$, $P < 0.001$) and their interaction was also significant ($t = -2.156$, $P < 0.031$). *Diabrotica* spp. damage was significantly increased in GLV-exposed, compared to control plants ($t = 4.740$, $P < 0.001$). When comparing each group of plants following their distance from the GLV dispenser, *Diabrotica* damage was generally higher on GLV-exposed plants (Fig. 4E–H). In field 2, 5 days after placing the dispensers (Fig. 4E), there was a trend ($F_{1,424} = 3.32$, $P < 0.07$) towards more damage on GLV-exposed plants than on control plants. The main difference was observed at 0.3–1.0 m distance from the dispenser ($F_{1,141} = 7.1485$, $P < 0.008$). After 8 days (Fig. 4F), the overall difference in *Diabrotica* spp. damage scores between GLV-exposed and control plants was stronger ($F_{1,424} = 6.27$, $P < 0.013$), mainly due to higher scores for GLV-exposed plants at <0,1 m ($F_{1,70} = 4.26$, $P < 0.043$). A similar overall effect was observed in the third field. After 5 days (Fig. 4G), significantly more *Diabrotica* spp. damage was observed on plants exposed to dispensers ($F_{1,142} = 4.25$, $P < 0.041$). After 8 days (Fig. 4H), the difference between GLV-exposed and control plants had increased ($F_{1,430} = 11.70$, $P < 0.001$), mainly at <0.1 m ($F_{1,142} = 6.76$, $P < 0.01$) and 0.3–1.0 m ($F_{1,142} = 8.08$, $P < 0.005$). However, there was no significant distance effect on *Diabrotica* spp. feeding damage, compared to the plants at <0.1 m from the GLV-dispenser ($t = 1.534$, $P < 0.125$ at 0.1–0.3 m and $t = 0.467$, $P < 0.641$ at 0.3–1.0 m). The *Diabrotica* spp. feeding damage varied significantly between the three fields (multivariate linear model (lm): $t = -12.43$, $P < 0.001$) and sampling day ($t = -4.145$, $P < 0.001$). There was a significant interaction between fields and sampling day ($t = 10.76$, $P < 0.001$).

2.4. Herbivore abundance

In field 1 (Fig. 6A), there were significantly more *S. frugiperda*-infested plants in GLV-exposed plots at a distance of 0.1–1.0 m (GLM, $P < 0.042$), than in control plots. This increased infestation was observed neither in field 2 (Fig. 6B) nor in field 3 (Fig. 6C). Infestation (number of larvae per plant) by *S. frugiperda* varied significantly between the three fields (Fig. 6A–C). In field 2, the infestation was significantly higher than in the two other fields (GLM, $P < 0.009$), but there was no difference between field 1 and field 3 (GLM, $P < 0.128$). The tendency towards higher *Diabrotica* spp. damage

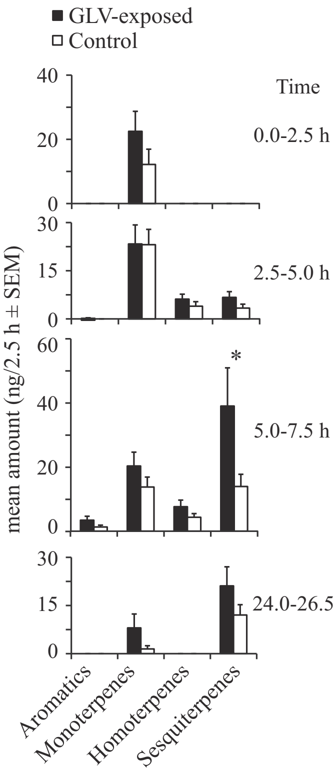

Fig. 3. Mean amount (ng/2.5h_SEM) of volatile compounds emitted by GLV-exposed and control maize plants (c.v. Delprim), upon mechanical damage. Emission was measured at four different sampling periods after induction (A) 0.0–2.5 h, (B) 2.5–5.0 h, (C) 5.0–7.5 h, and (D) 24.0–26.5 h). Data was analyzed using one-way analysis of variance (ANOVA). *$P < 0.05$.

in GLV-exposed plants was also observed when counting the adults on the plants (Fig. 6D–G). There were significantly more *Diabrotica* spp. adults on GLV-exposed plants than in control plants ($t = 3.658$, $P < 0.001$). In field 2, 5 days after placing the dispensers (Fig. 6D), there was an overall trend towards more *Diabrotica* beetles on GLV-exposed plants than in control plants ($F_{1,424} = 2.79$, $P < 0.096$). This difference was most evident at 0.1–0.3 m distance from the dispenser ($F_{1,137} = 4.05$, $P < 0.046$). Eight days after placing the dispensers (Fig. 6E), the difference in numbers of *Diabrotica* increased. There were significantly more adults on GLV-exposed plants than on control plants ($F_{1,212} = 7.49$, $P < 0.007$), particularly at <0.1 m ($F_{1,70} = 6.60$, $P < 0.012$). In field 3, the effect of the GLVs on the number of *Diabrotica* was slightly lower. Five days after placing the dispensers (Fig. 6F), a difference could be observed at <0.1 m ($F_{1,142} = 5.49$, $P < 0.020$). After 8 days (Fig. 6G), there was a trend towards more *Diabrotica* beetles on GLV-exposed than on control plants ($F_{1,430} = 3.033$, $P < 0.082$), particularly at 0.1–0.3 m from the dispenser ($F_{1,142} = 4.22$, $P < 0.042$). Overall, the distance had also a significant effect on the number of *Diabrotica*, compared with the plants at <0.1 m from the dispenser ($t = 2.861$, $P < 0.004$ for 0.1–0.3 m and $t = 5.221$, $P < 0.001$ for 0.3–1.0 m). The field had also a significant effect ($t = -6.428$, $P < 0.001$). In fields 2 and 3, we also counted aphids and planthoppers (Fig. 6H–K). As they were present only 5 days after placing the dispenser, the factors scoring day and the interaction (scoring day × field) were removed in the linear model. The exposure to GLVs tended to increase the number of aphids ($t = 1.894$, $P < 0.059$). Indeed, in field 2, 5 days after placing the dispensers (Fig. 6H), an overall trend towards more aphids on plants with dispensers could be observed ($F_{1,424} = 3.47$, $P < 0.063$). In field 3 (Fig. 6I), this trend was only observed at a distance of 0.3–1.0 m from the dispenser ($F_{1,142} = 2.78$, $P < 0.097$). There was a significant difference between fields in the number of aphids

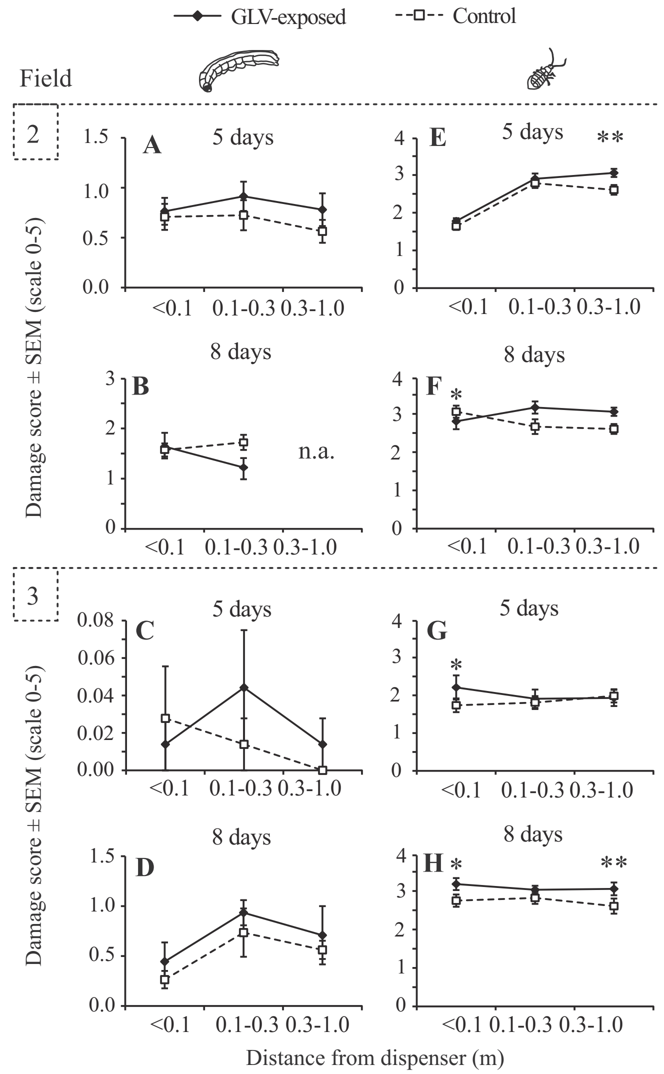

Fig. 4. Herbivore damage (scale 0–5) by *Diabrotica* spp. beetles (A–D) and *S. frugipera* larvae (E–H) on GLV-exposed and control maize plants. Herbivory was scored in two fields (2 and 3) 5 and 8 days after placing the dispensers. Plants at <0.1 m, 0.1–0.3 m and 0.3–1.0 m were score separately. Data were analyzed with one-way analysis of variance (ANOVA). *$P < 0.05$, **$P < 0.01$.

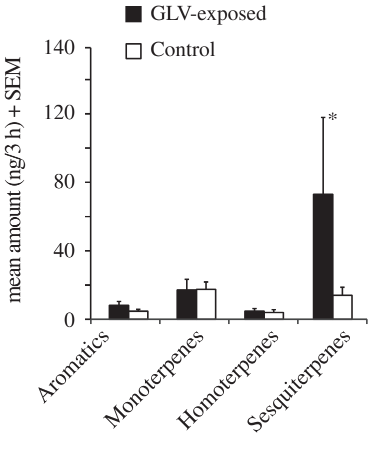

Fig. 5. Mean amount (ng/3 h ± SEM) of volatile compounds emitted by GLV-exposed and control maize plants (c.v. Tuxpeño Sequía) in the field. Data was analyzed with Wilcoxon pair-wise comparison. *$P < 0.05$.

counted on the plants ($t = -5.698$, $P < 0.001$). Planthoppers were also counted only on day five (Fig. 6J and K). In field 3, more planthoppers were found on control plants than on GLV-exposed plants at 0.1–0.3 m ($F_{1,142} = 4.17$, $P < 0.042$) There were significantly more planthoppers found in field 2 than in field 3 ($t = -7.857$, $P < 0.001$).

2.5. Parasitism

Overall, *S. frugiperda* was parasitized by seven species of parasitoids (Table 1). Two egg–larval parasitoids, *Chelonus insularis* (Cresson) (Hymenoptera: Braconidae) and *Chelonus cautus* (Cresson) (Hymenoptera: Braconidae), and five larval parasitoids, *Eiphosoma vitticolle* (Cresson) (Hymenoptera: Ichneumonidae), *C. marginiventris* (Cresson) (Hymenoptera:Braconidae), *Campoletis sonorensis* (Cameron) (Hymenoptera: Ichneumonidae), *Pristomerus spinator* (Fabricius) (Hymenoptera: Ichneumonidae). The species *Aleiodes laphygmae* (Viereck) (Hymenoptera: Braconidae) and Tachinidae were found only in summer 2008 (Field 1). In field 3, the parasitism by *C. cautus* was significantly lower on GLV-exposed plants than on control plants (GLM, $P < 0.027$), whereas *C. insularis* tended to be more attracted by GLV-exposed plants than by control plants at a distance of 0.1–0.3 m (GLM, $P < 0.082$). Unfortunately, due to a high mortality of the parasitoid larvae collected in field 1, only few individuals of *P. spinator*, *Eiphosoma viticolle*, Tachinidae, and *Aleoides* spp. could be identified from this field and therefore they are not included in Table 1.

2.6. Predation

The average number of recollected caterpillars on plants that were covered by a net was 2.67 ± 0.21 for control and 1.50 ± 0.72 for GLV-treated plants ($V = 13.5$, $P < 0.134$) (Fig. 7). The average number of recollected caterpillars on plants that were left uncovered was 0.8 ± 0.40 for control plants and 0.50 ± 0.34 for GLV-exposed plants (Wilcoxon signed rank test: $V = 4$, $P < 0.773$). There were significantly fewer *S. frugiperda* larvae recovered on uncovered control plants than on covered control plants ($P < 0.007$). However, there was no difference between uncovered and covered

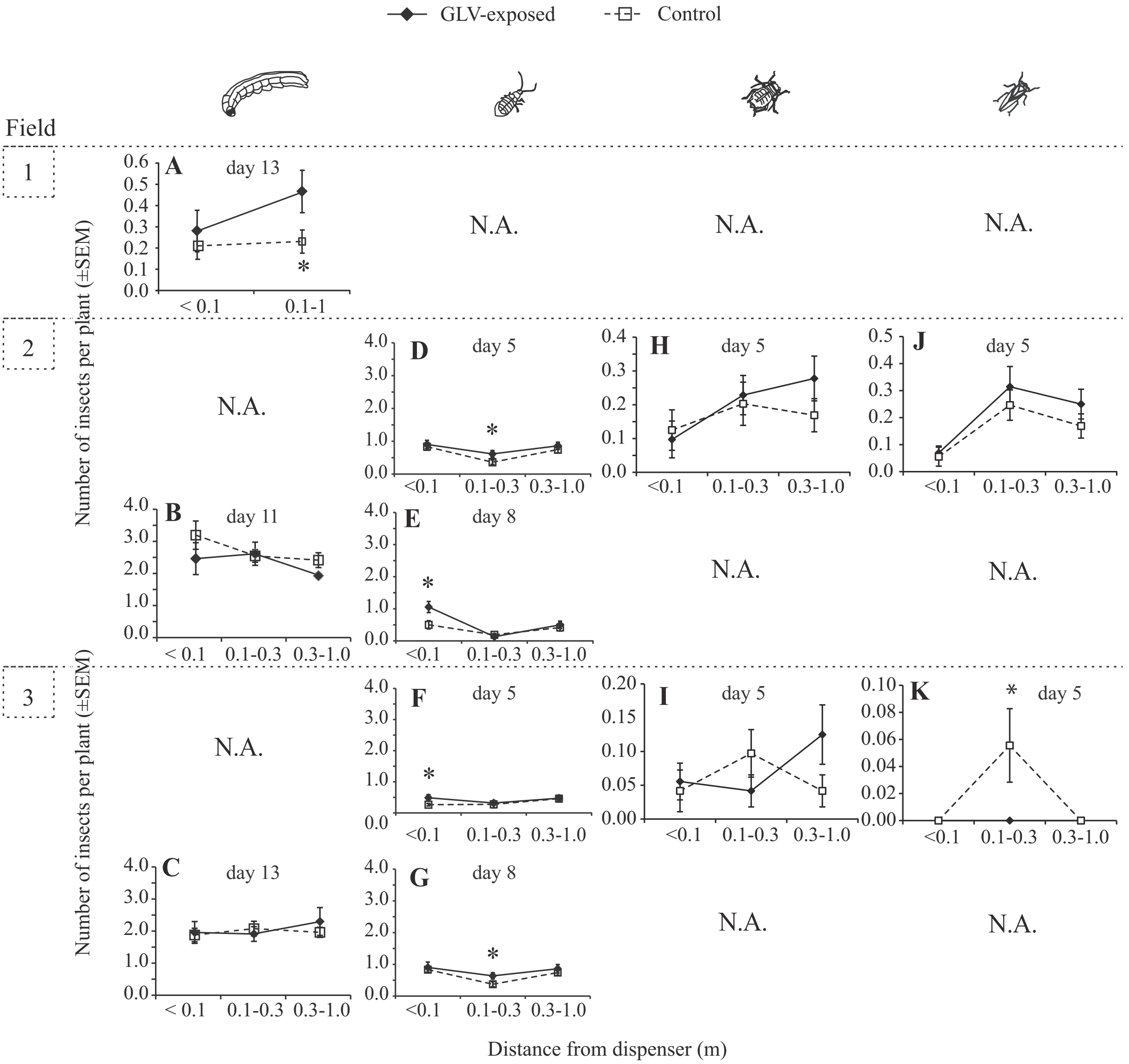

Fig. 6. Number of herbivores on GLV-exposed and control plants. Insects were counted in three fields. *S. frugiperda* (A–C) were counted 10–13 days after placing the dispensers in all fields. *Diabrotica* beetles (D–G) were counted in field 2 and 3, 5 and 8 days after placing the dispensers. Aphids (H and J) and planthoppers (J and K) were counted in field 2 and 3 5 days after placing the dispensers. Insects were counted separately on plants placed <0.1, 0.1–0.3, 0.3–1.0 m from the dispenser, except for field 1, where only two distances were collected separately. Data were analyzed with one-way analysis of variance (ANOVA). *$P < 0.05$), **$P < 0.01$.

Table 1
Parasitism of *Spodoptera frugiperda* caterpillars (number of parasitoids retrieved per collected *S. frugiperda*) in%. Parasitoids hatched from caterpillars collected from GLV-exposed and control plants depending on the distance from the dispenser (<0.1 m, 0.1–0.3 m and 0.3–1.0 m). An asterisk indicates a significant difference ($P < 0.05$), GLM with quasi-binomial correction; a dot indicates a statistical trend ($P < 0.10$).

	Field 2		Field 3	
	GLV-exposed ± SEM	Control ± SEM	GLV-exposed ± SEM	Control ± SEM
Total Parasitism	37.14 ± 4.79	36.98 ± 2.73	38.58 ± 4.66	37.86 ± 5.18
<0.1 m	42.37 ± 10.41	42.04 ± 2.40	45.21 ± 4.53	50.43 ± 9.65
0.1–0.3 m	41.25 ± 5.74	37.83 ± 6.01	43.99 ± 9.43	37.01 ± 11.43
0.3–1.0 m	35.25 ± 4.86	35.32 ± 2.04	32.46 ± 4.87	34.94 ± 5.47
Chelonus insularis	26.47 ± 3.56	27.23 ± 2.97	24.18 ± 2.86	21.70 ± 3.61
<0.1 m	28.59 ± 7.61	32.36 ± 3.59	29.61 ± 3.85	31.48 ± 6.93
0.1–0.3 m	33.53 ± 3.31	27.03 ± 7.27	**29.04 ± 5.37**	**20.57 ± 4.89**•
0.3–1.0 m	23.40 ± 3.89	25.71 ± 1.95	19.58 ± 4.03	20.53 ± 4.81
Eiphosoma vitticolle	7.05 ± 0.92	6.36 ± 0.65	6.35 ± 1.49	7.17 ± 1.29
<0.1 m	11.03 ± 3.97	5.75 ± 2.39	8.14 ± 2.82	10.03 ± 3.92
0.1–0.3 m	3.90 ± 1.56	6.55 ± 2.03	6.08 ± 2.39	7.86 ± 3.48
0.3–1.0 m	8.03 ± 0.96	6.32 ± 0.78	5.43 ± 1.33	5.84 ± 2.72
Pristomerus spinator	1.44 ± 0.69	0.90 ± 0.31	5.38 ± 1.63	4.79 ± 1.89
<0.1 m	2.08 ± 2.08	0.72 ± 0.72	4.42 ± 2.49	0.00 ± 0.00
0.1–0.3 m	0.93 ± 0.93	1.19 ± 0.78	6.62 ± 3.71	6.62 ± 3.71
0.3–1.0 m	1.50 ± 0.53	0.93 ± 0.34	5.50 ± 1.57	3.31 ± 1.72
Campoletis sonorensis	1.05 ± 0.54	1.40 ± 0.41	2.36 ± 0.77	2.76 ± 1.42
<0.1 m	0.67 ± 0.67	0.54 ± 0.54	3.05 ± 2.34	4.75 ± 2.23
0.1–0.3 m	1.43 ± 1.06	1.87 ± 0.86	2.24 ± 1.65	0.00 ± 0.00
0.3–1.0 m	0.85 ± 0.38	1.52 ± 0.58	1.44 ± 0.77	3.81 ± 1.85
Chelonus cautus	0.28 ± 0.19	0.56 ± 0.46	**0.31 ± 0.31**	**1.45 ± 1.45**∗
<0.1 m	1.39 ± 1.39	0.60 ± 0.60	0.00 ± 0.00	4.17 ± 4.17
0.1–0.3 m	0.00 ± 0.00	1.11 ± 0.71	0.00 ± 0.00	0.00 ± 0.00
0.3–1.0 m	0.17 ± 0.17	0.42 ± 0.42	0.51 ± 0.51	1.45 ± 1.45
Cotesia marginiventris	1.01 ± 0.47	0.83 ± 0.22	0.00±.0.00	0.00 ± 0.00
<0.1 m	0.00 ± 0.00	2.68 ± 2.05	0.00 ± 0.00	0.00 ± 0.00
0.1–0.3 m	1.11 ± 1.11	0.48 ± 0.48	0.00 ± 0.00	0.00 ± 0.00
0.3–1.0 m	1.48 ± 0.81	0.85 ± 0.33	0.00 ± 0.00	0.00 ± 0.00

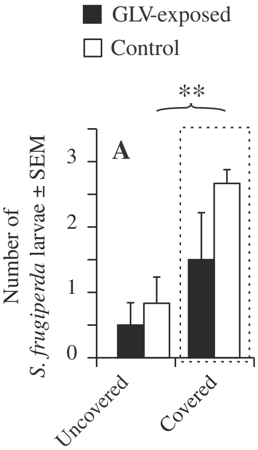

Fig. 7. Number of *S. frugiperda* larvae ± SEM retrieved from uncovered and netted plants in GLVexposed and control plants 12 days after artificial infestation with first-instar larvae.

GLV-exposed plants ($P < 0.276$). Thus, the predation rate of *S. frugiperda* caterpillars was estimated at 69.4 ± 13.9% for control plants and 66.7 ± 33.3% for GLV-exposed plants.

3. Discussion

In this study we investigated the effect of synthetic GLVs on the defence responses of maize plants and on the biological control of insect pests feeding on the plants. In the laboratory we found that maize plants exposed to synthetic GLVs responded with a stronger and faster emission of sesquiterpenes upon subsequent induction of the VOC-emission in these plants compared to plants that were not exposed the GLVs (Fig. 3). These results confirm findings from earlier studies that show that exposure to GLVs enhances the inducible emission of VOCs in plants (Arimura et al., 2001; Engelberth et al., 2004; Frost et al., 2008). This so-called priming effect (Ton et al., 2007) might also be important in the field, as insect-damaged maize plants emitted higher amounts of sesquiterpenes after exposure to synthetic GLVs (Fig. 5). However, as these plants were exposed to various biotic and abiotic stresses, including herbivore damage, immediately after germination, the higher emission of sesquiterpenes in the field was probably not just the result of priming, but rather a synergistic effect of GLVs on the emission of these compounds. Indeed, earlier studies have shown that at high concentrations GLVs directly affect the emission of sesquiterpenes (Farag et al., 2005; Ruther and Furstenau, 2005; Ruther and Kleier, 2005). Our study appears to be the first field study to show that physiological realistic concentrations of GLVs (Fig. 1) are sufficient to alter the VOC emission in GLV-exposed plants.

Based on a correlation between sesquiterpene emission and jasmonic acid-related defence mechanisms (Schmelz et al., 2003) we hypothesised that plants with increased sesquiterpenes emission would also better resist herbivore attack. However, we could not confirm this in our field experiments. On the contrary, plants that were exposed to GLVs generally suffered increased herbivore damage (Fig. 4) and higher numbers of several herbivores were counted on theses plants (Fig. 6), as compared to control plants. This could be the result of attraction of these herbivores towards GLVs and/or the higher amount of sesquiterpenes emitted by GLV-exposed plants. Coleoptera, in particular are known to be attracted to herbivore-induced volatiles (Halitschke et al., 2008; Loughrin et al., 1995; Bolter et al., 1997), including GLVs (Ruther and Hilker, 2003) and sesquiterpenes (Hammack, 2001). Indeed, the increased herbivore presence and herbivory was most significant for *Diabrotica* beetles, which is in agreement with evidence that they are directly attracted to sesquiterpenes (Hammack, 2001). Adults of lepidopteran species, however, are usually repelled by induced VOCs (De Moraes et al., 2001; Landolt, 1993; Huang et al., 2009), including adult *S. frugiperda* females, which have been reported to be repelled by maize VOCs induced by conspecific larvae in cage experiments (De Moraes et al., 2001). Yet, the larvae of *S. frugiperda* are attracted to inducible VOCs (Carroll et al., 2006), which could be one explanation for the somewhat higher presence of *S. frugiperda* larvae on GLV-exposed maize plants than on control plants. For other herbivores, such as aphids and plant hoppers, there was no clear effect of GLV exposure, which is somewhat in contrast to an earlier study that showed that aphids avoid induced plants (Bernasconi et al., 1998).

Almost 40% of the collected *S. frugiperda* larvae were parasitized. The parasitoids included *C. insularis*, *E. vitticolle*, *P. spinator*, *C. sonorensis*, *C. cautus*, and *C. marginiventris* (Table 1), confirming earlier studies that parasitism of this pest is high in the subtropical area of Mexico (Hoballah et al., 2004; Jourdie et al., 2008; Molina-Ochoa et al., 2003, 2004). As there is plenty of evidence from laboratory and field studies that GLVs are attractive to parasitoids (Degenhardt, 2009; Hoballah and Turlings, 2005; Whitman and Eller, 1990; Bruce et al., 2010) we had expected higher parasitism rates of *S. frugiperda* in plots with GLV-exposed plants. This was not the case. A possible explanation might be the simultaneous higher emission of sesquiterpenes by GLV-exposed plants, which might repel certain parasitoids. In fact, the innate attraction of two important parasitoids of *Spodoptera* species is stronger to blends with low terpenoid emissions (D'Alessandro and Turlings, 2005; D'Alessandro et al., 2009), and sesquiterpenes only become important as attractants after the wasps have associated these compounds with host presence (Schnee et al., 2006). Another explanation for a lack of increased parasitism rates in our study may be the fact that the main parasitoid was the egg–larval parasitoid *C. insularis*. GLVs and other volatiles that are associated with herbivory are less likely to attract parasitoids in search of insect eggs and may even be repellent as indicated by the lower numbers of another egg–larval parasitoid, *C. cautus*, recovered from larvae on GLV-exposed plants (Table 1).

It should also be noted that the caterpillars were collected irrespective of their developmental stage, with a large portion of early developmental stages, i.e. first or second instar. Early stages can only be parasitized by a restricted number of species and at a later stage could have been parasitized by other primary parasitoids and/or hyperparasitoids (Marktl et al., 2002; McDonald and Kok, 1991; Tamò et al., 2006). If we had collected more larvae at later stages this might have resulted in a different parasitoid species composition.

From the additional experiment with potted maize plants we estimated that as much as two thirds of the caterpillars on a maize plant may be lost through predation (Fig. 7). There was no significant effect of synthetic GLVs on predation, despite the fact that predator attraction in the field can be enhanced with synthetic VOCs (James, 2003b).

Finally, the effects of GLVs depended on the distance from the dispensers. This is in agreement with the results from Lee (2010), who also show that the effect of methyl salicylate in strawberry plots is dependent on the distance from the dispenser. In these types of studies it is important to work with realistic dosages of the applied compounds, as at high concentrations a volatile foraging cue that normally is an attractant can become a repellent (Snoeren et al., 2010). Since the GLV exposure affected the emission of several VOCs that might be involved in the insects' foraging behaviour, the overall effects on attraction and repellency might have been blurred.

4. Conclusions

In conclusion, although some authors suggest that GLVs are sufficient to improve resistance against herbivores and pathogens (Shiojiri et al., 2006) and to enhance the attraction of both parasitoids and predatory insects (James, 2003a; James and Price, 2004) our study suggests that the opposite may occur as well. In the field, GLVs enhanced the number of some herbivore species and barely had an effect on parasitism rates. The results of our study further confirm that GLVs are physiologically active at very low concentrations and also affect the emission of the VOCs in GLV-exposed plants. It is important that such physiological changes are also considered in studying the potential of using VOCs to attract biological control agents.

5. Experimental

5.1. GLV-dispensers

Amber glass vials (2 ml, Supelco, Sigma Aldrich, Buchs, Switzerland) were filled with 100 mg of glass wool, that were first washed with dichloromethane and heated for 16 h at 250 °C. They were then loaded with 0.2 ml of a mixture of 80% (*Z*)-3-hexenal (>92.5% purity, (NEAT) in 50% triacetin for stability, Bedoukian Research Inc., USA), 10% (*Z*)-3-hexenol (>98% GC, Sigma–Aldrich, Buchs, Switzerland), 8% (*Z*)-3-hexenyl acetate (>98%, SAFC Supply Solutions, St. Louis, MO, USA), and 2% (*E*)-2-hexenal (>99%, ACROS Organics, New Jersey, USA). The vials were closed with open screw caps that contained a rubber septum. The rubber septa were pierced with a 2 μl microcapillary (Drummond) and the vials were then wrapped in aluminium foil for heat-protection and to avoid photodegradation of the GLVs (Fig. 1). In the laboratory we collected volatiles from the dispenser 4 days after preparations and found only small shifts in ratios that are minor in comparison with the variability that can be expected in the field. Similarly prepared empty dispensers were used as controls.

5.2. Volatile collection in the laboratory

To measure the amounts of GLVs emitted by the dispensers, the 12 dispensers were placed each into an empty volatile collection bottle and the emitted volatiles were collected and analyzed as described by D'Alessandro and Turlings (2005) (Fig. 1). To confirm that GLVs released from the dispenser prime maize plants, we performed a laboratory experiment with young maize plants under standardized conditions. Six nine-day-old maize plants (c.v. Delprim) were exposed to GLV-dispensers for 16 h inside a glass bottle with purified, humid air flowing through at a rate of 0.3 l/min passing. After this exposure, the plants were removed from the glass bottles, damaged mechanically and 10 μl *S. littoralis* regurgitant was applied to the wounds (for details, see Turlings et al., 1998b). The plants were then placed in clean glass bottles, with an air flow of 1.2 l/min. VOCs were sampled and analyzed as described by Ton et al. (2007) with a sampling time of 2.5 h at four time-points: 0–2.5 h, 2.5–5.0 h, 5.0–7.5 h, and 24.0–26.5 h. Control plants were not exposed to GLVs, but otherwise were treated and sampled similarly. This experiment was repeated twice.

5.3. Volatile collection in the field

The amounts of GLVs emitted by the dispensers in the field were measured by attaching the dispenser to a 50 cm long metal wire, which was stuck into the soil next to an assigned maize plant in the experimental plots, placing the microcapillary close to the whorl of the plant (<10 cm). Twelve maize plants with GLV-dispensers or with empty control dispensers were covered with a 50 cm Nalophan sleeve (Omya AG, Oftringen, Switzerland, 150 mm diameter). At the bottom, the sleeve was closed with a plastic seal below the oldest leaf and at the top it was attached to a metal wire, to prevent mechanical damage due to wind moving the sleeve. A tubular glass device (23 × 17 × 12 mm) with a screw cap was attached to the bottom of the bag (as described by Turlings et al., 1998a). Through this device a SuperQ filter (Analytical Research Systems, Inc., Gainesville FL, USA) was inserted into the bag. Air was pulled through the filter tube with the use of an air-sampling pump (SKC 222 series, Blanc Labo S.A., Lonay, Switzerland) for 3 h at 0.6 l/min. For each of 12 replications, VOCs were collected simultaneously from a GLV-exposed plant and from a control plant. Plants were checked for presence of herbivores before placing them inside the Nalophan sleeve. The VOCs were eluted from the filters and analyzed as described by D'Alessandro and Turlings (2005). Variations in release rates between summer 2008 and winter 2009 can be partly explained by differences in temperatures (maxima 34 ± 1 °C in summer 2008 vs 28 ± 1 °C in winter 2009; minima 25 ± 1 °C in summer 2008 and 15 ± 1 °C in winter 2009).

5.4. Maize fields and plants

Maize plots were planted with the variety "Tuxpeño Sequía" at the Ernest W. Sprague research station in Agua Fría, in the state of Puebla, Mexico (20°26′56.93″N, 97°38′23.98″ W, 98 masl). Three fields were sown on three dates (Field 1 on 16th June 2008, field 2 on 6th February 2009, and field 3 on 11th February 2009) in 30 rows of 25 m length, 70 cm between rows and 20 cm between plants within the rows. The field was divided into 12 plots of 10 rows by 4 m, leaving 2 m as a buffer between plots within the same row (Fig. 2). Three to four days after germination (V2–V3 leaf stage, collar of the second-third leaf visible), plants were counted and checked for insect damage. Subsequently a GLV-dispenser was placed next to 72 plants in GLV-exposed plots, and a control dispenser next to 72 plants in control plots, as indicated in Fig. 2.

5.5. Herbivores and herbivore damage

Herbivores and herbivore damage were measured at different distances from the dispensers. In field 1, two distances were sampled (<0.1 m and 0.1–1.0 m). In field 2 and 3, three distances were considered (<0.1 m, 0.1–0.3 m, and 0.3–1.0 m). Herbivore damage was measured by rating the feeding damage on the leaves by the chrysomelid adults *Diabrotica* spp. and flea beetles (*Chaetocnema* spp.), and by *S. frugiperda* on maize plants, using a scale from 0 to 5 (0 = absence of feeding, 1 = few small feeding spots, 2 = several feeding spots, 3 = spread feeding spots, 4 = severely damaged plant, 5 = dead plant) (after Wiseman et al., 1966; Montes et al., 1996). At the same time, herbivore numbers of these insects, of aphids (Sternorrhyncha: Aphidoidea) and of planthoppers (Hemiptera: Fulgoromorpha) present on the plants were recorded. The field plants were scored for insect damage and numbers of shoot herbivores in fields 2 and 3. Scorings and counts were performed on 2 days (day 5 and day 8 after placing dispensers). Finally, the plants were dissected in the field on day 12–13 after placing the volatile dispensers, and all caterpillars were retrieved from the plants. The caterpillars were counted and placed individually in single compartments of 24-well ELISA plates (Jourdie et al., 2008) containing artificial, maize-based diet (Hoballah et al., 2004). In addition, we recorded the number of plants that were infested by *S. frugiperda*, by *Diatraea saccharalis* (Lepidoptera: Pyralidae), or by both of these herbivores. These were the only two Lepidoptera species found on the maize plants of that developmental stage in the field.

5.6. Parasitoids

Caterpillars of *S. frugiperda* were collected in the field 10–13 days after the plants were first exposed to the GLV dispensers. The insects were collected and reared separately, depending on the distance from the GLV dispenser, as described above. The collected caterpillars were placed on a maize-based diet (Hoballah et al., 2004) in 24-well ELISA plates (Jourdie et al., 2008) and reared until adult moths emerged or the parasitoid larvae emerged from their hosts. All parasitoid larvae and pupae were removed, placed in a Petri dish and reared out to adulthood. Parasitoids were identified using an identification manual (Cave, 1995).

5.7. Predation

Predation was measured in a separate field experiment with potted maize plants. For this purpose, 36 potted maize plants (c.v. Tuxpeño Sequía) were sown in 10 l plastic pots in the greenhouse. At the four-leaf stage, they were placed in a maize field with distances of 2 m between pots. Eighteen plants were exposed to GLV dispensers and 18 to an empty control dispenser, and placed in pairs on the border of a field, each pair consisting of two GLV-exposed plants, or two control plants. All plants were then infested with 45 ± 5 first instar *S. frugiperda* larvae, mixed with grits (Wiseman et al., 1980). One plant of each pair was covered with an insect-proof net of 1 mm nylon sleeve, analogous to predation studies in wheat (Schmidt et al., 2003). On top, the net was tied close and attached to a stake and on the bottom it was fastened to the pot with a rubber band, to prevent soil-born predators from entering. After a period of 14 days, the sleeves were removed; the caterpillars were then retrieved and counted on each plant. Predation was calculated as the ratio (%) of retrieved larvae in uncovered plants to larvae retrieved in covered plants.

5.8. Statistical analysis

All the statistical analyses in this study were performed using the R programming environment (R Development Core Team, 2010). Laboratory volatile data were analyzed using *F*-statistics in one-way analysis of variance (ANOVA). Field volatile data were analyzed using pairwise *t*-test for each treatment and its respective control (paired by sampling day). The Wilcoxon signed rank test was applied when uniformity of variances was not met. The herbivory data (*Diabrotica* spp. adults feeding scores and insect number per plant, aphid number per plant, planthopper number per plant, and *S. frugiperda* damage score) were analyzed in a linear model with the effect of the field, the effect of the scoring day, the effect of the dispenser and the effect of the distance from the dispenser. For each distance (<0.1 m, 0.1–0.3 m, and 0.3–1.0 m) and time-point (two and three), the data was then compared with one-way analysis of variance (ANOVA). The number of herbivores was tested using ANOVA or the Kruskal–Wallis rank sum test if assumptions were not met. The herbivore infestation data were analyzed as binomial data in a generalised linear model (GLM), with plants either infested or not. However, overdispersed data required to adapt the statistical method, as suggested by Verdon et al. (2007). The difference between fields and between scoring days was tested with a quasi-binomial distribution in a GLM. Parasitization data were analyzed in a similar way as the herbivore data in a GLM with quasi-binomial distribution, *S. frugiperda* larvae being either parasitized or not. Predation data were analyzed in a pair-wise *t*-test by comparing the ratio of the number of larvae recovered from uncovered plants and the number of larvae recovered from covered plants, in GLV-exposed and control plants.

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References

Allmann, S., Baldwin, I.T., 2010. Insects betray themselves in nature to predators by rapid isomerization of green leaf volatiles. Science 329 (5995), 1075–1078.

Arimura, G., Matsui, K., Takabayashi, J., 2009. Chemical and molecular ecology of herbivore-induced plant volatiles: proximate factors and their ultimate functions. Plant and Cell Physiology 50 (5), 911–923.

Arimura, G., Ozawa, R., Horiuchi, J., Nishioka, T., Takabayashi, J., 2001. Plant–plant interactions mediated by volatiles emitted from plants infested by spider mites. Biochemical Systematics and Ecology 29 (10), 1049–1061.

Arimura, G.-I., Koepke, S., Kunert, M., Volpe, V., David, A., Brand, P., Dabrowska, P., Maffei, M.E., Boland, W., 2008. Effects of feeding *Spodoptera littoralis* on lima bean leaves: IV. Diurnal and nocturnal damage differentially initiate plant volatile emission. Plant Physiology 146 (3), 965–973.

Baldwin, I.T., 2010. Plant volatiles. Current Biology 20 (9), 392–397.

Bernasconi, M.L., Turlings, T.C.J., Ambrosetti, L., Bassetti, P., Dorn, S., 1998. Herbivore-induced emissions of maize volatiles repel the corn leaf aphid, shape *Rhopalosiphum maidis*. Entomologia Experimentalis et Applicata 87 (2), 133–142.

Bolter, C.J., Dicke, M., Van Loon, J.J.A., Visser, J.H., Posthumus, M.A., 1997. Attraction of Colorado potato beetle to herbivore-damaged plants during herbivory and after its termination. Journal of Chemical Ecology 23 (4), 1003–1023.

Broz, A.K., Broeckling, C.D., De-la Pena, C., Lewis, M.R., Greene, E., Callaway, R.M., Sumner, L.W., Vivanco, J.M., 2010. Plant neighbor identity influences plant biochemistry and physiology related to defense. BMC Plant Biology 10, 115.

Bruce, T.J.A., Midega, C.A.O., Birkett, M.A., Pickett, J.A., Khan, Z.R., 2010. Is quality more important than quantity? Insect behavioural responses to changes in a volatile blend after stemborer oviposition on an African grass. Biology letters 6 (3), 314–317.

Bruce, T.J.A., Wadhams, L.J., Woodcock, C.M., 2005. Insect host location: a volatile situation. Trends in Plant Science 10 (6), 269–274.

Bruinsma, M., van Broekhoven, S., Poelman, E.H., Posthumus, M.A., Muller, M.J., van Loon, J.J.A., Dicke, M., 2010. Inhibition of lipoxygenase affects induction of both direct and indirect plant defences against herbivorous insects. Oecologia 162 (2), 393–404.

Carroll, M.J., Schmelz, E.A., Meagher, R.L., Teal, P.E.A., 2006. Attraction of *Spodoptera frugiperda* larvae to volatiles from herbivore-damaged maize seedlings. Journal of Chemical Ecology 32 (9), 1911–1924.

Cave, R.D., 1995. Manual para el reconocimiento de parasitoides de plagas agrícolas en América Central. Zamorano Academic Press, El Zamorano.

Choh, Y., Shimoda, T., Ozawa, R., Dicke, M., Takabayashi, J., 2004. Exposure of lima bean leaves to volatiles from herbivore-induced conspecific plants results in emission of carnivore attractants: active or passive process? Journal of Chemical Ecology 30, 1797–1808.

D'Alessandro, M., Brunner, V., von Mérey, G.E., Turlings, T., 2009. Strong attraction of the parasitoid *Cotesia marginiventris* towards minor volatile compounds of maize. Journal of Chemical Ecology 35 (9), 999–1008.

D'Alessandro, M., Turlings, T.C.J., 2006. Advances and challenges in the identification of volatiles that mediate interactions among plants and arthropods. Analyst 131 (1), 24–32.

D'Alessandro, M., Turlings, T.C., 2005. In-situ modification of herbivore-induced plant odors: a novel approach to study the attractiveness of volatile organic compounds to parasitic wasps. Chemical Senses 30, 739–753.

D'Auria, J.C., Pichersky, E., Schaub, A., Hansel, A., Gershenzon, J., 2007. Characterization of a BAHD acyltransferase responsible for producing the green leaf volatile (*Z*)-3-hexen-1-yl acetate in *Arabidopsis thaliana*. Plant Journal 49 (2), 194–207.

De Moraes, C.M., Mescher, M.C., Tumlinson, J.H., 2001. Caterpillar-induced nocturnal plant volatiles repel conspecific females. Nature 410 (6828), 577–580.

Degenhardt, J., 2009. Indirect defense responses to herbivory in grasses. Plant Physiology 149 (1), 96–102.

Dicke, M., van Poecke, R.M., de Boer, J.G., 2003. Inducible indirect defence of plants: from mechanisms to ecological functions. Basic and Applied Ecology 4 (1), 27–42.

Engelberth, J., Alborn, H.T., Schmelz, E.A., Tumlinson, J.H., 2004. Airborne signals prime plants against insect herbivore attack. Proceedings of the National Academy of Sciences of the United States of America 101 (6), 1781–1785.

Engelberth, J., Seidl-Adams, I., Schultz, J.C., Tumlinson, J.H., 2007. Insect elicitors and exposure to green leafy volatiles differentially upregulate major octadecanoids and transcripts of 12-oxo phytodienoic acid reductases in *Zea mays*. Molecular Plant–Microbe Interactions 20 (6), 707–716.

Farag, M., Fokar, M., Abd, H., Zhang, H., Allen, R., Paré, P., 2005. (*Z*)-3-hexenol induces defense genes and downstream metabolites in maize. Planta 220 (6), 900–909.

Farag, M., Pare, P., 2002. C-6-green leaf volatiles trigger local and systemic VOC emissions in tomato. Phytochemistry 61 (5), 545–554.

Frost, C.J., Mescher, M.C., Dervinis, C., Davis, J.M., Carlson, J.E., De Moraes, C.M., 2008. Priming defense genes and metabolites in hybrid poplar by the green leaf volatile *cis*-3-hexenyl acetate. New Phytologist 180 (3), 722–733.

Halitschke, R., Stenberg, J.A., Kessler, D., Kessler, A., Baldwin, I.T., 2008. Shared signals – 'Alarm calls' from plants increase apparency to herbivores and their enemies in nature. Ecology Letters 11 (1), 24–34.

Hammack, L., 2001. Single and blended maize volatiles as attractants for diabroticite corn rootworm beetles. Journal of Chemical Ecology 27 (7), 1373–1390.

Hansson, B.S., Larsson, M.C., Leal, W.S., 1999. Green leaf volatile-detecting olfactory receptor neurones display very high sensitivity and specificity in a scarab beetle. Physiological Entomology 24 (2), 121–126.

Heil, M., 2008. Indirect defence via tritrophic interactions. New Phytologist 178 (1), 41–61.

Heil, M., Kost, C., 2006. Priming of indirect defences. Ecology Letters 9 (7), 813–817.

Heil, M., Ton, J., 2008. Long-distance signalling in plant defence. Trends in Plant Science 13 (6), 264–272.

Heil, M., Walters, D.R., 2009. Ecological consequences of plant defence signalling. Plant Innate Immunity 51, 667–716.

Himanen, S.J., Blande, J.D., Klemola, T., Pulkkinen, J., Heijari, J., Holopainen, J.K., 2010. Birch (*Betula* spp.) leaves adsorb and re-release volatiles specific to neighbouring plants – A mechanism for associational herbivore resistance? New Phytologist 186 (3), 722–732.

Hoballah, M.E., Degen, T., Bergvinson, D., Savidan, A., Tamò, C., Turlings, T.C., 2004. Occurrence and direct control potential of parasitoids and predators of the fall armyworm (Lepidoptera: Noctuidae) on maize in the subtropical lowlands of Mexico. Agricultural and Forest Entomology 6, 83–88.

Hoballah, M.E., Turlings, T.C.J., 2005. The role of fresh versus old leaf damage in the attraction of parasitic wasps to herbivore-induced maize volatiles. Journal of Chemical Ecology 31 (9), 2003–2018.

Huang, C.-H., Yan, F.-M., Byers, J.A., Wang, R.-J., Xu, C.-R., 2009. Volatiles induced by the larvae of the Asian corn borer (*Ostrinia furnacalis*) in maize plants affect behavior of conspecific larvae and female adults. Insect Science 16 (4), 311–320.

James, D.G., 2003a. Field evaluation of herbivore-induced plant volatiles as attractants for beneficial insects: methyl salicylate and the green lacewing, *Chrysopa nigricornis*. Journal of Chemical Ecology 29 (7), 1601–1609.

James, D.G., 2003b. Synthetic herbivore-induced plant volatiles as field attractants for beneficial insects. Environmental Entomology 32 (5), 977–982.

James, D.G., Grasswitz, T.R., 2005. Synthetic herbivore-induced plant volatiles increase field captures of parasitic wasps. BioControl 50 (6), 871–880.

James, D.G., Price, T.S., 2004. Field-testing of methyl salicylate for recruitment and retention of beneficial insects in grapes and hops. Journal of Chemical Ecology 30 (8), 1613–1628.

Jourdie, V., Alvarez, N., Turlings, T.C.J., 2008. Identification of seven species of hymenopteran parasitoids of *Spodoptera frugiperda*, using polymerase chain reaction amplification and restriction enzyme digestion. Agricultural and Forest Entomology 10 (2), 129–136.

Karban, R., 2010. Neighbors affect resistance to herbivory – A new mechanism. New Phytologist 186 (3), 565–566.

Kessler, A., Halitschke, R., Diezel, C., Baldwin, I.T., 2006. Priming of plant defense responses in nature by airborne signaling between *Artemisia tridentata* and *Nicotiana attenuata*. Oecologia 148 (2), 280–292.

Landolt, P.J., 1993. Effects of host plant leaf damage on cabbage-looper moth attraction and oviposition. Entomologia Experimentalis Et Applicata 67 (1), 79–85.

Larsson, M.C., Leal, W.S., Hansson, B.S., 2001. Olfactory receptor neurons detecting plant odours and male volatiles in *Anomala cuprea* beetles (Coleoptera: Scarabaeidae). Journal of Insect Physiology 47 (9), 1065–1076.

Lee, J.C., 2010. Effect of methyl salicylate-based lures on beneficial and pest arthropods in strawberry. Environmental Entomology 39 (2), 653–660.

Loughrin, J.H., Potter, D.A., Hamilton-Kemp, T.R., 1995. Volatile compounds induced by herbivory act as aggregation kairomones for the Japanese-beetle (*Popillia japonica* Newman). Journal of Chemical Ecology 21 (10), 1457–1467.

Mäntylä, E., Alessio, G.A., Blande, J.D., Heijari, J., Holopainen, J.K., Laaksonen, T., Piirtola, P., Klemola, T., 2008. From plants to birds: Higher avian predation rates in trees responding to insect herbivory. Plos One 3 (7).

Marktl, R.C., Stauffer, C., Schopf, A., 2002. Interspecific competition between the braconid endoparasitoids *Glyptapanteles porthetriae* and *Glyptapanteles liparidis* in *Lymantria dispar* larvae. Entomologia Experimentalis Et Applicata 105 (2), 97–109.

McDonald, R.C., Kok, L.T., 1991. Hyperparasites attacking *Cotesia glomerata* (L.) and *Cotesia rubecula* (Marshall) (Hymenoptera: Braconidae) in Southwestern Virginia. Biological Control 1 (2), 170–175.

Molina-Ochoa, J., Carpenter, J.E., Heinrichs, E.A., Foster, J.E., 2003. Parasitoids and parasites of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the Americas and Caribbean basin: an inventory. Florida Entomologist 86 (3), 254–289.

Molina-Ochoa, J., Carpenter, J.E., Lezama-Gutierrez, R., Foster, J.E., Gonzalez-Ramirez, M., Angel-Sahagun, C.A., Farias-Larios, J., 2004. Natural distribution of hymenopteran parasitoids of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae in Mexico. Florida Entomologist 87 (4), 461–472.

Montes, J.I.L., Pitre, H.N., Meckenstock, D.H., Gomez, F., 1996. Evaluation of a sorghum population for resistance to fall armyworm (Lepidoptera: Nocuitdae). Tropical Agriculture 73 (1), 43–48.

Mumm, R., Hilker, M., 2005. The significance of background odour for an egg parasitoid to detect plants with host eggs. Chemical Senses 30, 337–343.

Paré, P.W., Tumlinson, J.H., 1999. Plant volatiles as a defense against insect herbivores. Plant Physiology 121 (2), 325–332.

Ruther, J., Furstenau, B., 2005. Emission of herbivore-induced volatiles in absence of a herbivore – Response of *Zea mays* to green leaf volatiles and terpenoids. Zeitschrift Fur Naturforschung C-a Journal of Biosciences 60 (9–10), 743–756.

Ruther, J., Hilker, M., 2003. Attraction of forest cockchafer Melolontha hippocastani to (*Z*)-3-hexen-1-ol and 1,4-benzoquinone: application aspects. Entomologia Experimentalis et Applicata 107 (2), 141–147.

Ruther, J., Kleier, S., 2005. Plant–plant signaling: ethylene synergizes volatile emission in *Zea mays* induced by exposure to (*Z*)-3-hexen-1-ol. Journal of Chemical Ecology 31 (9), 2217–2222.

Schmelz, E., Alborn, H., Banchio, E., Tumlinson, J., 2003. Quantitative relationships between induced jasmonic acid levels and volatile emission in *Zea mays* during *Spodoptera exigua* herbivory. Planta 216 (4), 665–673.

Schmidt, M.H., Lauer, A., Purtauf, T., Thies, C., Schaefer, M., Tscharntke, T., 2003. Relative importance of predators and parasitoids for cereal aphid control. Proceedings of the Royal Society B: Biological Sciences 270 (1527), 1905–1909.

Schnee, C., Kollner, T.G., Held, M., Turlings, T.C.J., Gershenzon, J., Degenhardt, J., 2006. The products of a single maize sesquiterpene synthase form a volatile defense signal that attracts natural enemies of maize herbivores. PNAS 103 (4), 1129–1134.

Shiojiri, K., Kishimoto, K., Ozawa, R., Kugimiya, S., Urashimo, S., Arimura, G., Horiuchi, J., Nishioka, T., Matsui, K., Takabayashi, J., 2006. Changing green leaf volatile biosynthesis in plants: An approach for improving plant resistance against both herbivores and pathogens. Proceedings of the National Academy of Sciences 103 (45), 16672–16676.

Snoeren, T.A.L., Mumm, R., Poelman, E.H., Yang, Y., Pichersky, E., Dicke, M., 2010. The herbivore-induced plant volatile methyl salicylate negatively affects attraction of the parasitoid *Diadegma semiclausum*. Journal of Chemical Ecology 36 (5), 479–489.

Tamò, C., Roelfstra, L.-L., Guillaume, S., Turlings, T.C., 2006. Odour-mediated long-range avoidance of interspecific competition by a solitary endoparasitoid: a time-saving foraging strategy. Journal of Animal Ecology.

Takabayashi, J., Dicke, M., Takahashi, S., Posthumus, M.A., 1994. Leaf age affects composition of herbivore-induced synomones and attraction of predatory mites. Journal of Chemical Ecology 20, 373–386.

R Development Core Team, 2010. R: a language and environment for statistical computing.

Ton, J., D'Alessandro, M., Jourdie, V., Jakab, G., Karlen, D., Held, M., Mauch-Mani, B., Turlings, T.C.J., 2007. Priming by airborne signals boosts direct and indirect resistance in maize. Plant Journal 49 (1), 16–26.

Turlings, T.C., Wäckers, F., 2004. Recruitment of predator and parasitoid by herbivore-injured plants. Advances in Insect Chemical Ecology 2, 21–75.

Turlings, T.C.J., Bernasconi, M., Bertossa, R., Bigler, F., Caloz, G., Dorn, S., 1998a. The induction of volatile emissions in maize by three herbivore species with different feeding habits: possible consequences for their natural enemies. Biological Control 11, 122–129.

Turlings, T.C.J., Lengwiler, U.B., Bernasconi, M.L., Wechsler, D., 1998b. Timing of induced volatile emissions in maize seedlings. Planta 207 (1), 146–152.

Turlings, T.C.J., Tumlinson, J.H., Lewis, W.J., 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. Science 250 (4985), 1251–1253.

Unsicker, S.B., Kunert, G., Gershenzon, J., 2009. Protective perfumes: the role of vegetative volatiles in plant defense against herbivores. Current Opinion in Plant Biology 12 (4), 479–485.

Verdon, A., Margraf, N., Davison, A.C., Rahier, M., Naisbit, R.E., 2007. Conserved oviposition preferences in alpine leaf beetle populations despite host shifts and isolation. Ecological Entomology 32 (1), 62–69.

Walling, L.L., 2000. The myriad plant responses to herbivores. Journal of Plant Growth Regulation 19 (2), 195–216.

Whitman, D.W., Eller, F.J., 1990. Parasitic wasps orient to green leaf volatiles. Chemoecology 1 (2), 69–76.

Wiseman, B.R., Davis, F.M., Campbell, J.E., 1980. Mechanical infestation device used in fall armyworm plant-resistance programs (Lepidoptera, Noctuidae). Florida Entomologist 63 (4), 425–432.

Wiseman, B.R., Painter, R.H., Wassom, C.E., 1966. Detecting corn seedling differences in greenhouse by visual classification of damage by fall armyworm. Journal of Economic Entomology 59 (5), 1211–1214.

Yi, H.-S., Heil, M., Adame-Alvarez, R.M., Ballhorn, D.J., Ryu, C.-M., 2009. Airborne induction and priming of plant defenses against a bacterial pathogen. Plant Physiology 151 (4), 2152–2161.

Zeringue, H.J., 1992. Effects of C-6–C-10 alkenals and alkanals on eliciting a defense response in the developing cotton boll. Phytochemistry 31 (7), 2305–2308.

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