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RESEARCH ARTICLE

Specific response to herbivore-induced *de novo* synthesized plant volatiles provides reliable information for host plant selection in a moth

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SUMMARY

Animals depend on reliable sensory information for accurate behavioural decisions. For herbivorous insects it is crucial to find host plants for feeding and reproduction, and these insects must be able to differentiate suitable from unsuitable plants. Volatiles are important cues for insect herbivores to assess host plant quality. It has previously been shown that female moths of the Egyptian cotton leafworm, *Spodoptera littoralis* (Lepidoptera: Noctuidae), avoid oviposition on damaged cotton *Gossypium hirsutum*, which may mediated by herbivore-induced plant volatiles (HIPVs). Among the HIPVs, some volatiles are released following any type of damage while others are synthesized *de novo* and released by the plants only in response to herbivore damage. In behavioural experiments we here show that oviposition by *S. littoralis* on undamaged cotton plants was reduced by adding volatiles collected from plants with ongoing herbivory. Gas chromatography–electroantennographic detection (GC–EAD) recordings revealed that antennae of mated *S. littoralis* females responded to 18 compounds from a collection of headspace volatiles of damaged cotton plants. Among these compounds, a blend of the seven *de novo* synthesized volatile compounds was found to reduce oviposition in *S. littoralis* on undamaged plants under both laboratory and ambient (field) conditions in Egypt. Volatile compounds that are not produced *de novo* by the plants did not affect oviposition. Our results show that ovipositing females respond specifically to the *de novo* synthesized volatiles released from plants under herbivore attack. We suggest that these volatiles provide reliable cues for ovipositing females to detect plants that could provide reduced quality food for their offspring and an increased risk of competition and predation.

Key words: gas chromatography–electroantennographic detection (GC–EAD), *Gossypium hirsutum*, herbivory, HIPVs, induced direct defence, olfaction, oviposition, repellents, signal reliability, *Spodoptera littoralis*.

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INTRODUCTION

The ability to detect and process information from the environment is fundamental for animals and they must constantly gather information to adjust their behaviour to environmental changes (Bukovinszky et al., 2008; Dall et al., 2005). Making decisions based on cues associated with resource quality has a large impact on reproductive fitness for vertebrates and invertebrates (Andersson and Simmons, 2006; Dall et al., 2005; Schmidt and Whelan, 2010). To efficiently guide behaviours, the cues used should be detectable and distinct from background noise to reduce the risk of mistakes (Zangerl, 2003). The response to sensory cues allows animals to respond to spatial heterogenity and temporal variation of resources and to make phenotypic changes that can increase fitness (Agrawal et al., 2006; Auld et al., 2010; Dangles et al., 2009).

Host plant selection in herbivorous insects involves the processing of different sensory cues from plants and other organisms associated with the plants (Schoonhoven et al., 2005). For many insects, survival of the progeny is largely dependent on the capacity of the female to find a suitable host. Negative effects on herbivore populations have been found when females select host plants that are of poor quality, i.e. low nutritional value, infested by other insects or potentially attractive to natural enemies (Bernays, 2001). Such factors exert a strong selection pressure on insects to distinguish between unsuitable and suitable host plants for feeding and reproduction (Agrawal et al., 2006; Hopkins et al., 2009). Thus, avoiding unsuitable plants, such as non-hosts or host plants of poor quality, can be as important as finding suitable host plants.

Volatile cues from plants are important for many herbivorous insects during host plant selection as they carry information about different plants in the environment (Bruce and Pickett, 2011). However, all plants emit volatiles, and the challenge for females is to detect and respond to reliable cues that indicate plant identity and quality. Herbivore-induced plant volatiles (HIPVs) are emitted after damage to plants and would represent an efficient signal to evaluate food resources for herbivores (Arimura et al., 2009; Dicke and Baldwin, 2010; Heil and Karban, 2010). By avoiding plants emitting HIPVs, herbivores can increase survival and fitness by reducing the consumption of plants with increased levels of secondary compounds, and increased risk for larval mortality, competition for food resources and attack by natural enemies (Karban, 2011). Repellency to HIPV-emitting plants has been found in a few insect herbivores (Bernasconi et al., 1998; De Moraes et al., 2001; Kessler and Baldwin, 2001). For herbivores, these volatile compounds can have both high detectability and high reliability as cues as they are usually emitted from the plant in large amounts and in specific ratios after herbivore damage to the plant (Dicke,

2009; Clavijo McCormick et al., 2012). Therefore, for herbivore insects, they represent an efficient signal to evaluate food resources (Arimura et al., 2009; Dicke and Baldwin, 2010; Heil and Karban, 2010).

Damaged cotton produces HIPVs both locally at the site of damage and systemically distal to damaged parts (Loughrin et al., 1994; McCall et al., 1994; Röse and Tumlinson, 2005). The compounds emitted locally are stored in the tissues and released rapidly in response to wounding or mechanical damage to the tissues, while other compounds are synthesized *de novo* by the plant and are released systemically in response to herbivore feeding (Paré and Tumlinson, 1997a). Production of the *de novo* synthesized compounds is induced by elicitors present in larval saliva and regurgitate (Alborn et al., 1997). The biosynthesis of *de novo* produced, herbivore-induced compounds and their effect on attraction of natural enemies of the herbivores has been investigated (Paré and Tumlinson, 1999). However, their importance in plant resistance, e.g. by affecting the herbivore host selection behaviour, has not been studied.

The Egyptian cotton leafworm, *Spodoptera littoralis* (Lepidoptera: Noctuidae) Boisduval 1833, is a generalist insect herbivore that utilizes a wide range of wild and cultivated plants, including cotton *Gossypium hirsutum*, in Africa and the Middle East (Brown and Dewhurst, 1975). Female *S. littoralis* have been found to reduce oviposition on cotton plants damaged by conspecific leaf-feeding larvae or a root-feeding heterospecific herbivore (Anderson and Alborn, 1999; Anderson et al., 2011). We have found that volatiles from cotton plants damaged by conspecifics repelled oviposition in *S. littoralis* (Zakir et al., 2013). Electrophysiological studies have identified highly sensitive and selective sensilla responding to plant volatiles in *S. littoralis* (Anderson et al., 1995; Anderson et al., 1996; Saveer et al., 2012) and among these are receptor neurons specifically responding to HIPVs emitted by damaged cotton plants (Jönsson and Anderson, 1999). However, not all volatiles from damaged plants reliably indicate the presence of insects on this plant. To make more accurate decisions, it would be advantageous for females to respond to volatiles indicating herbivore damage, and not to volatiles emitted only after mechanical damage.

In this study, we used behavioural and electrophysiological methods to identify the volatile compounds emitted from plants damaged by conspecifics that are repellent for ovipositing *S. littoralis* females. We hypothesized that *de novo* synthesized volatile compounds among HIPVs are sufficient to reduce oviposition. Systemic production of these volatile compounds would be a reliable indicator for the female to avoid plants already under attack by conspecific herbivores.

MATERIALS AND METHODS

Insects

The *S. littoralis* for the laboratory experiments were obtained from a culture established in 2007 from moths collected in the Alexandria region in Egypt. Wild insect material has been introduced into the culture at least once annually. The insects were reared on a potato-based semi-synthetic diet (Hinks and Byers, 1976) and all stages were kept at 25±2°C, 65±5% relative humidity (RH) and 16 h:8 h light:dark photoperiod. The sex of the pupae was determined under a microscope and the sexes were kept in separate emergence boxes until used in the experiments. Third–fourth instar larvae and 2 day old mated female moths were used in all experiments.

For the field experiments, male and female moths of *S. littoralis* were taken from a culture maintained at the Department of Zoology, Assiut University, Egypt. The insects were collected from the field to establish the culture in the laboratory. In the laboratory, the insects were reared on an artificial diet based on wheat germ and casein. The insects were reared at 25±1°C, ≥70% RH, and a 16 h:8 h light:dark photoperiod.

Plants

For the laboratory experiments, cotton seeds (*G. hirsutum* L., var. Delta pineland 90) were soaked overnight in water and planted individually in pots (diameter 14 cm) filled with soil and grown in a climatized greenhouse at 25±2°C and 65±5% RH. Artificial light (Philips, SON-T, 400 W), positioned 1–2 m above the plants was provided in addition to natural light. Plants with five to six true leaves were used for the experiments.

For the field experiments, cotton plants were grown in an experimental field (1400 m^2) near the city of Assiut, Egypt.

Headspace collection of HIPVs

Plants with ongoing larval feeding initiated 48 h earlier were placed in an odor collection chamber (25±5°C, 70±5% RH). Charcoal-filtered air was pumped (PM 10879-NMP30; KNF Neuberger, Stockholm, Sweden) at a flow rate of 600 ml min^{-1} through a clean (oven-burned overnight at 350°C) glass jar (5 l) starting 2 h before the HIPV collection to remove any remaining volatiles. To avoid mechanical injury and contamination from the soil, the green foliage of the plant was adjusted carefully inside the glass jar and the jar was placed on a glass plate separating pot from plant. The system was purged for 30–45 min with previously adjusted airflow before volatiles were collected at an outlet at the top of the jar on a glass filter filled with 150 mg of Super-Q adsorbent (80/100 mesh, Supelco, Sigma-Aldrich, St Louis, MO, USA) for 12 h at a flow rate of ~200 ml min^{-1}. Collections were made in darkness. Using two headspace setups, undamaged plants and plants with ongoing larval damaged were sampled (seven replicates per treatment) simultaneously. The volatiles were eluted from the filters with 500 µl hexane. None of the identified compounds have been identified from emissions from larvae and larval frass (Anderson et al., 1993) (P.A., unpublished).

Electrophysiology

The system used for gas chromatography–electroantennographic detection (GC–EAD) recording has been described previously (Jönsson and Anderson, 1999). Briefly, the recordings were performed with a modified Hewlett-Packard HP-6890A gas chromatograph (GC). Headspace sample (2 µl) was injected into the injector of the GC. Hydrogen was used as the carrier gas (50 cm s^{-1} linear velocity) and a capillary HP-innowax column (30 m×0.25 mm i.d.) with a stationary phase of 0.25 µm. A four-way column split adjusted at the end of the capillary HP-innowax column divided the effluent into two halves. One half passed through the flame ionization detector (FID) and other half was introduced into a glass tube (diameter 8 mm) with a charcoal-filtered and humidified air stream (flow ~1.5 l min^{-1}) flushing over the antennal preparation for EAD. The temperature of the injector, detector and EAD-outlet was 220, 250 and 220°C, respectively. Splitless injection was made at 30°C oven temperature. The temperature programme started after 2 min with a rate of 5°C min^{-1} up to 200°C. The FID and EAD signals were both monitored and analysed on a computer using GC-EAD software (Electro Antenno-Detection, Syntech, Hilversum, The Netherlands).

For recordings, one antenna from 2 day old mated female *S. littoralis* moths was excised at its base and two to three segments

from the distal part were cut off. The antenna was mounted between two glass capillary electrodes, filled with Ringer solution (Beadle–Ephrussi), into which Ag wires were inserted. One electrode was connected to ground and the other to a high impedance DC amplifier in a signal connection interface box (IDAC 2, Syntech). To test the quality of the antennal preparation, 2 µl of headspace samples was applied on a filter paper strip placed in a pipette and the solvent was allowed to evaporate for 30 s. The 2 µl from the pipette was injected into the air stream passing over the antennal preparation.

Chemicals

Compounds used in the laboratory and field oviposition experiments were diluted in redistilled *n*-hexane (LabScan, Gliwice, Poland). The purity and source of the compounds used was: (*Z*)-3-hexenal (98%), (*E*)-β-ocimene (~75%), (*Z*)-3-hexenyl acetate (98%), (*Z*)-3-hexenol (98%), β-caryophyllene (98%), α-humulene (98%), phenylethylalcohol (98%), (*E*)-nerolidol (98%) and indole (99%), all from Sigma-Aldrich; β-myrcene (95%) and (±)linalool (97%) from Fluka (Sigma-Aldrich); methyl jasmonate (98%) from SAFC (Sigma-Aldrich); (*E*)-2-hexenal (98%), (*E*,*E*)-α-farnesene (99%) and (*Z*)-jasmone (98%) from Bedoukian (Danbury, CT, USA); methyl benzoate (97%) from Acros Organics (Thermo Fisher Scientific, Geel, Belgium); (*E*)-2,4-dimethyl-1,3,7-nonatriene (DMNT, 99%, a gift from Prof. Wittko Francke, Hamburg, Germany); and (*E*,*E*)-2,4,6-trimethyl-1,3,7,11-tridecatetraene (TMTT, gift from Prof. Monika Hilker, Berlin, Germany).

Chemical analysis

For chemical analyses, samples from overnight headspace collections (2 µl) were injected manually on a combined gas chromatograph and mass spectrometer (GC–MS): 6890 GC and 5975 MS (both Agilent Technologies, Palo Alto, CA, USA). The GC was equipped with a 30 m×0.25 mm fused silica column coated with DB-Wax (polyethylene glycol, d_f=0.25 µm, Agilent Technologies). Temperature was programmed from 30°C (3 min hold) at 8°C min^{-1} to 225°C (10 min hold). The injector temperature was 225°C and the transfer line was kept at 150°C for 17 min, then heated at 8°C min^{-1} to 225°C to track the oven temperature. Helium was used as carrier gas, at 35 cm s^{-1}, and the electron impact (EI mode) mass spectra were obtained at 70 eV, scanning *m*/*z* 29–400, at 2 scans s^{-1}. Compounds were identified by coinjection of synthetic references or by comparison with commercially available MS libraries (NIST and Wiley) and published K_i values (www.pherobase.com). GC–EAD active compounds were quantified on the basis of their ion abundance relative to the ion abundance shown by the known amount of the added heptyl acetate as an internal standard.

Volatile compound delivery system

Glass jars (5 l) were used for exposure to volatiles (Fig. 1). A continuous stream of charcoal-filtered air ~80 ml min^{-1} was pushed by a pump (PM 10879-NMP30; KNF Neuberger) into the inlet of the jar and transferred from the outlet to the oviposition cage through a Teflon tube (i.d. 1.5 mm). In the cage, the Teflon tube with five to six small holes in the distal part was attached to the stem of an undamaged plant inside the oviposition cage. Thus, the headspace odour collected from the plant was released from the perforated Teflon tube along the stem of the undamaged plants placed in the oviposition cage.

For continuous production and exposure of HIPVs to the undamaged plants, 10–12 *S. littoralis* larvae were released on a plant and allowed to feed continuously over a period of 96 h before and then throughout the oviposition experiment. When older larvae stopped feeding on the leaves they were replaced with young ones.

Glass vials (2 ml) were used as dispensers under both laboratory and field conditions for release of collected headspace extracts and synthetic compound mixtures (Fig. 2). The top of the Teflon tube with a cotton wick was exposed to the air, while the end was inserted into the vial, through a hole in the lid, and dipped into the collected headspace extract or synthetic mixture of compounds. The hole in the lid was slightly larger than the diameter of the Teflon tube to avoid varying air pressure inside the vial. The vials maintained known and continuous release rates of the compounds through the cotton wick placed inside the Teflon tube (i.d. 1.5 mm) over the experimental period. The release rate from the tube could be altered by the the length of the cotton wick that was extruded from the top of the Teflon tube. Before the start of each experiment, the release rate from the cotton wick was measured by monitoring the amount of released solvent (without active compounds) during the time of the oviposition test. The concentration of the extract or compound mixture solution was then adjusted to correspond to the release rate from damaged cotton plants. The volatiles emitted from the damaged plants and from the Teflon tubes with either headspace extract or the synthetic mixtures were collected for each treatment. GC analysis showed that the emissions of individual compounds of the different treatments differed maximally by 5% from the emission from plants with feeding larvae.

Oviposition experiments: laboratory

The laboratory experiments were performed in cages (120×80×60 cm) kept in a climatized greenhouse chamber with an internal ambient airflow, 25±2°C and 65±5% RH. In addition to normal daylight, the greenhouse experimental chamber was illuminated from 06.00 h till 18.00 h by artificial light (Philips, SON-T, 400 W). An undamaged cotton plant was placed on one side and another undamaged cotton plant was placed on the other side of the

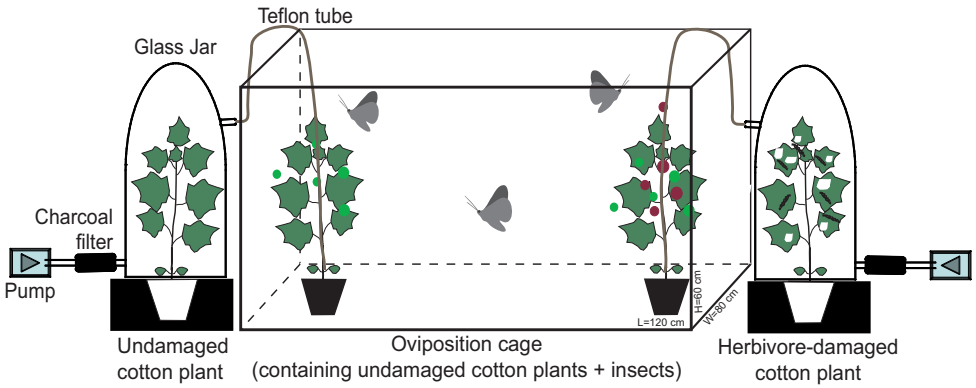

Fig. 1. Laboratory oviposition experiment setup. Undamaged plants were placed inside the cage with one plant at each end, 80 cm apart. Undamaged plant volatiles or hexane (control) was supplied to the undamaged plant on one side, while either herbivore-induced plant volatiles (HIPVs) or mixtures of synthetic compounds were supplied to the other plant.

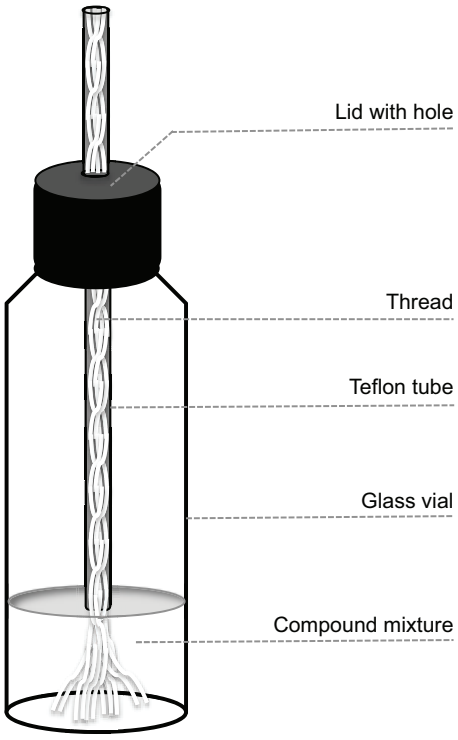

Fig. 2. Glass vial used as dispenser for headspace collections and synthetic compound mixtures.

cage and the distance between the plants was around 80 cm (Fig. 1). These plants were attached to the volatile compound delivery system from outside the cage. Eight female and 10 male *S. littoralis* moths, with no prior experience of cotton volatiles, were released in a perforated plastic box (24×18×7 cm) for mating. The box was placed inside the oviposition cages in the presence of undamaged plants and the moths were released 2 h after the start of release of the added volatile compounds. The moths were allowed to lay eggs on the plants during the scotophase and the egg batches were collected each morning for three successive days from the plants in the experiments using plant-collected volatiles and headspace collection and 1 day for the experiments with the synthetic compounds.

Five different oviposition experiments were performed in the laboratory. In the first experiment, female *S. littoralis* moths were allowed to choose between undamaged cotton plants with volatiles added from either a plant with on-going damage by *S. littoralis* larvae or an undamaged plant. For the subsequent experiments, volatiles were only added to one of the undamaged plants in the cage and the other plant served as the control plant, with hexane solvent added only. In the second experiment, headspace collection from cotton plants with ongoing larval feeding was tested.

In the next series of experiments the moths were offered a choice between undamaged control plants and undamaged plants with added synthetic compound mixtures. The release of compound at the plant was adjusted, by altering how much the wick protruded from the Teflon coating, to attain the same proportion and concentration of synthetic compounds found in the headspace collections made during the night. In the third experiment, a synthetic mixture of 18 GC–EAD active compounds was tested. Subsequently, in the fourth experiment a synthetic mixture of the seven compounds known as *de novo* produced after herbivore damage was tested. Lastly, in the fifth experiment a mixture of the remaining 11 compounds of the 18-compound mixture was tested. In the experiments both in the laboratory and under field conditions, the egg batches were weighed on an analytical scale (mg), and the number of eggs calculated (no. of eggs=mass of eggs×20).

Oviposition experiments: field

The experiments were performed in cages (140×110×90 cm). The cages were placed in pairs with the short sides (110 cm width) facing each other and were separated by a distance of about 50 cm (Fig. 3). The minimum distance between different pairs of cages was 12 m, over the field area of about 1400 m^2. Six cages in three pairs (i.e. two cages at a time) were used in the first trial, which started on the 3 September 2011, and another replicate of six cages in three pairs was set up on 15 September 2011, making a total sample size of 12 cages. In the first trial, field-grown plants were covered with the cages and two potted plants with a synthetic mixture of *de novo* compounds were placed between the cages; another two potted plants with hexane solvent alone were placed outside the far end of each cage in the pair (Fig. 3). In the second trial, the positions of the plants exposed to synthetic compounds *versus* hexane solvent were interchanged, to minimize the directional wind drift effects, although the wind speed in this region of Egypt is very stable during the night (Sadek et al., 2010). The area inside each cage was divided into two zones at the start of the experiment, one close to the side of the exposed plants and the other close to the side of the unexposed plants. Ten female and 8 male newly emerged moths, reared on artificial diet in the laboratory, were introduced into each cage. All the plants in each area were checked daily for egg batches for at least 10 days after the appearance of the first egg batch. Because of the abundant use of pesticides in the area where the experiments were performed, the population of herbivores is very low and thus the plants used

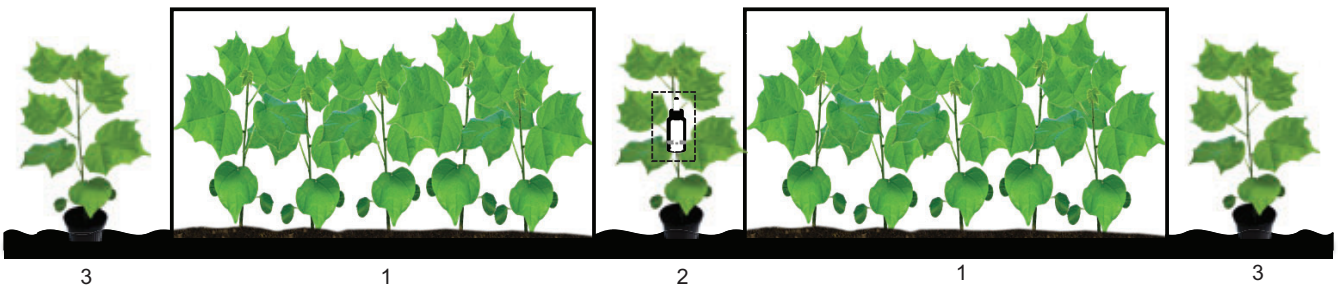

Fig. 3. Field oviposition experiment set-up. Two groups of undamaged field-cultivated plants (1), around 25 cm apart, were covered with cages and moths were released into these (cages) for oviposition. Outside the oviposition cage, undamaged plants with glass vials containing synthetic compounds (2) or undamaged cotton plants supplied with hexane solvent alone (3) were placed adjacent to test plants inside the oviposition cage.

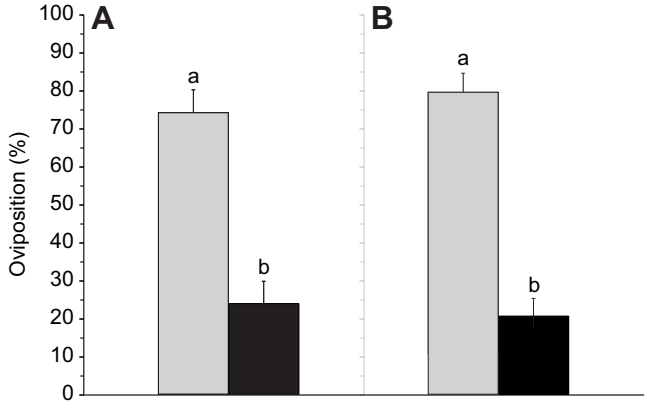

Fig. 4. Oviposition on undamaged cotton plants with added volatiles from cotton plants (mean + s.e.m.). The percentage of eggs after adding (A) volatile emissions directly from damaged (black) *versus* undamaged (grey) cotton plants, and (B) headspace collections from damaged cotton plants (black) *versus* hexane control (grey). Wilcoxon signed rank test for paired differences was used for statistical analysis; different letters show significant differences at $P<0.05$.

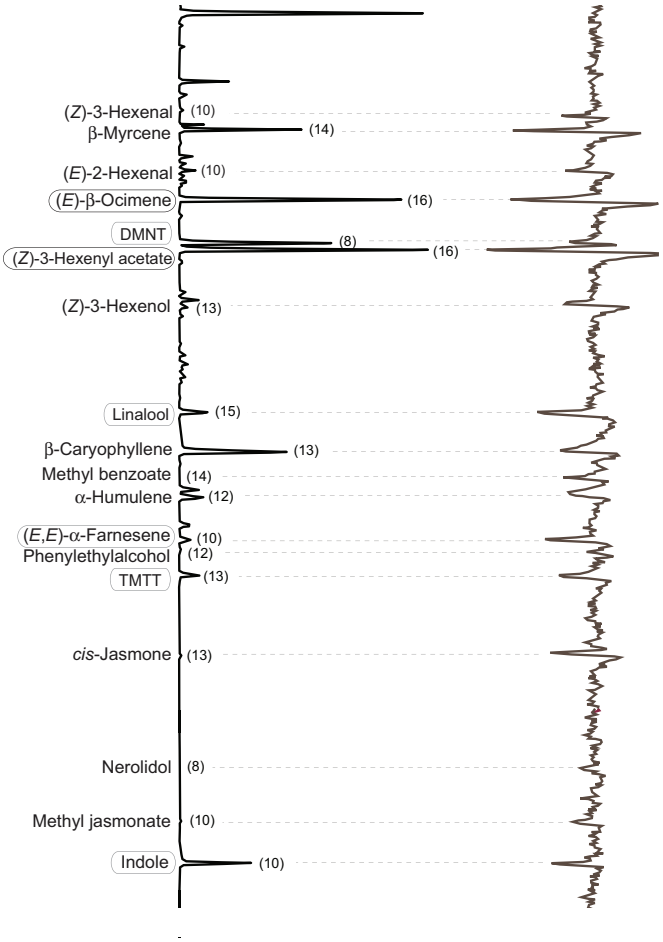

Fig. 5. Averaged gas chromatography–electroantennographic detection (GC–EAD) trace of responses from antennae of mated female *S. littoralis* moths (right-hand trace) to headspace collections of cotton plants damaged by the larvae of *S. littoralis* (left-hand trace). The number of responses to the compounds identified by gas chromatography–mass spectrometry is given on the right-hand side of the GC-trace. The circled compounds represent the seven *de novo* GC–EAD active compounds found.

in the experiments had very little (if any) visible damage by herbivores during the experiments.

Data analysis

The effect of HIPVs and a synthetic mimic of GC–EAD-active compounds was calculated in terms of oviposition by female *S. littoralis* moths on undamaged cotton plants, both in laboratory experiments and under field conditions, and was analysed by using Wilcoxon signed rank test for paired differences. For the analysis, the mass of the individual egg batches collected was used. All statistical analyses were performed using Minitab 16 software (Minitab Inc., v. 16.1.0.0). The level of significance for oviposition preference was selected as $\alpha=0.05$. Microsoft Office Excel 2008 software and Adobe Illustrator CS4 2008 were used for calculations and graphical representation of the data.

RESULTS

Female moths of *S. littoralis* laid more eggs (63 egg batches in total) on the side of the cage with added volatiles from an undamaged plant (75%) than on the side with volatiles from a damaged plant (25%) (Wilcoxon signed rank test for paired differences; $N=12$, $P=0.005$) (Fig. 4A). When the headspace extract was added to one side, the females laid a significantly higher number of eggs (32 egg batches) on the control side (79%) ($N=8$, $P=0.03$) compared with the side with headspace volatiles (21%) (Fig. 4B).

GC–EAD analyses of headspace collections ($N=16$) of HIPVs showed that excised antennae of mated female *S. littoralis* moths were able to detect 18 volatile compounds in total, released by the damaged cotton plant (Fig. 5). Responses to *E*-β-ocimene, (*Z*)-3-hexenyl, linalool, β-myrcene and methyl benzoate were found in nearly all recordings, while responses to other compounds were not found as frequently, as a result of varying sensitivity between the recordings. We set the threshold for considering the compounds as active to responses in at least half of the recordings (eight recordings).

In the experiment in which the synthetic mixture of all 18 GC–EAD active compounds was used, the mated females laid a low percentage of eggs (30 egg batches) on the undamaged cotton plant with this mixture added (14%) ($N=8$, $P=0.008$) compared with the side with added hexane solvent (86%) (Fig. 6A). The females also laid fewer eggs (36 egg batches) on the undamaged cotton plant with an added synthetic mixture of the seven *de novo* GC–EAD active compounds found (23%) ($N=12$, $P=0.004$) when compared with plants with hexane solvent added (77%) (Fig. 6B). In contrast, females showed no difference in egg laying (44 egg batches) on the undamaged cotton plant with the 11-compound mixture added (45%) ($N=8$, $P=0.56$) and on plants with hexane solvent added (55%) (Fig. 6C). Also, under field conditions females laid fewer eggs (221 egg batches) on plants with added *de novo* compounds (39%) ($N=10$, $P=0.03$) compared with the plants with hexane solvent added (61%) (Fig. 6D).

DISCUSSION

The emission of HIPVs from herbivore-damaged cotton plants reduced oviposition in *S. littoralis*. The females laid fewer eggs on undamaged cotton plants when odours from plants with ongoing damage by conspecific larvae were added. Our study further demonstrates that of 18 compounds released by herbivore-damaged cotton that elicit an antennal response, a blend of seven compounds *de novo* produced after herbivore damage reduced oviposition in *S. littoralis*. In contrast, a blend of the remaining 11 compounds that

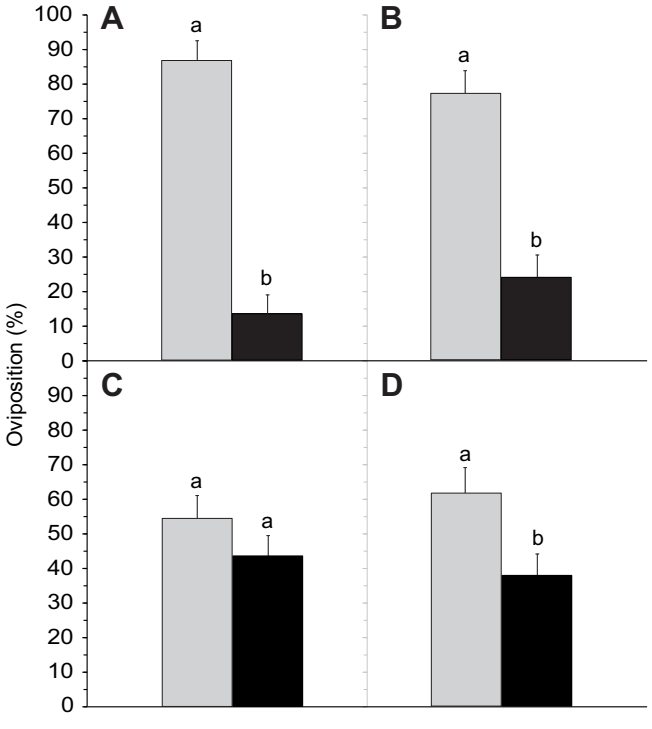

Fig. 6. *Spodoptera littoralis* oviposition preference for undamaged cotton plants (mean + s.e.m.) after adding synthetic volatile compounds (black) *versus* hexane control (grey) to undamaged neighbouring plants. In laboratory experiments, synthetic mixtures of (A) all 18 GC–EAD active compounds, (B) seven *de novo* produced compounds and (C) 11 compounds that are not *de novo* produced were used. Under field conditions, (D) the blend of seven *de novo* synthesized compounds was used. Wilcoxon signed rank test for paired differences was used for statistical analysis; different letters show a significant effect on plant selection at $P<0.05$.

are released after damage, but that are not *de novo* produced after herbivore damage, did not affect egg distribution. This shows that during host plant selection, ovipositing females of *S. littoralis* respond specifically to volatiles with high information value and reliability for assessing plant suitability. The presence of conspecifics is used in animal systems as an indicator of resource quality and the level of competition, but also to reduce costs for sampling (Dall et al., 2005; Pasqualone and Davis, 2011). In cotton, feeding by *S. littoralis* larvae induces both a large quantitative increase and a qualitative change in the emission of volatiles (Loughrin et al., 1994; McCall et al., 1994). The higher amount of volatiles emitted after damage increases the detectability of herbivore-damaged cotton plants for females searching for a suitable ovipostion site. The concentration of volatiles around the plant, and consequently the distance from the plant at which they can be detected, increases. All HIPVs increase the detectability of a damaged plant. However, it is only the compounds that are *de novo* produced after herbivore damage that fulfill the criteria for cues needed to make well-informed decisions (Schmidt et al., 2009; Valone, 2007; Vet and Dicke, 1992). They have high detectability, as cotton plants systemically produce high amounts of these compounds some hours after herbivore attack (Loughrin et al., 1994; McCall et al., 1994). In addition, they have high reliability as they are only produced after herbivore damage (Paré and Tumlinson, 1997b; Röse et al., 1998).

In an environment of high uncertainty, such as heterogeneous habitats, the importance of reliable cues increases (Agrawal et al., 2006; McLinn and Stephens, 2010). For an ovipositing female in a polyphagous insect, such as *S. littoralis*, environmental uncertainty may lead to errors in decision making and can have effects on fitness, for example through prolonged search time or increased predation risk (Munoz and Blumstein, 2012). Compounds that are produced *de novo* after herbivore damage provide reliable cues with high informational value through their high specificity that can increase signal to noise ratio and thus reduce uncertainty about the environment during host plant search (Dall et al., 2005). HIPVs may serve as reliable cues to detect not only conspecifics but also other herbivore species attacking the host plant that are hard to detect, such as below-ground herbivores (Erb et al., 2008; Rasmann et al., 2005). In *S. littoralis*, females have been shown to avoid oviposition on cotton plants that are attacked by root-feeding wireworm larvae (Anderson et al., 2011), suggesting a systemic response in the above-ground parts of the plant to the root-feeding herbivore that includes HIPVs. Furthermore, we have shown that HIPVs from cotton can provide associational resistance to neighbouring plants and affect the attractiveness not only of the emitting plant but also of other nearby interspecific or intraspecific plants (Zakir et al., 2013). This could influence herbivore distribution of eggs and dispersal between plants, but potentially also between plant patches.

By avoiding infested plants, *S. littoralis* females can better locate plants that provide good conditions for their progeny. Oviposition on an undamaged plant will initially provide the newly emerged larvae with food containing lower concentrations of defence compounds, which will increase their survival and development rate (Anderson and Agrell, 2005; Bezemer et al., 2004). Oviposition on previously undamaged plants also minimizes the risk of exploitative competition for and depletion of food resources. Furthermore, the larvae will experience reduced cannibalism or predation. Lastly, the exposure to natural enemies, such as parasitic wasps, is decreased on initially undamaged plants as herbivore-damaged plants are known to release volatile cues used by natural predators (Dicke and Baldwin, 2010). Thus, *de novo* synthesized compounds allow females to evaluate several aspects of host plant quality at a distance.

There are a few studies that have shown that damage-induced compounds repel oviposition in herbivores. For instance, in tobacco plants, herbivore-induced volatiles were found to be repellent for ovipositing moths in both laboratory and field conditions (De Moraes et al., 2001; Kessler and Baldwin, 2001). For female *Heliothis virescens* moths, oviposition was reduced on undamaged tobacco plants when they were exposed to a mixture of five volatile compounds from herbivore-damaged tobacco plants released during the scotophase (De Moraes et al., 2001). Lastly, female *Manduca* moths avoided tobacco plants in the field during oviposition when treated with linalool in the same ratios as released by damaged tobacco plants (Kessler and Baldwin, 2001).

For the plant, the production and release of *de novo* HIPVs can be beneficial in several ways. As we have shown in this study, the *de novo* produced compounds signal herbivore presence and by affecting herbivore behaviour reduce the risk of additional attack by herbivores. Furthermore, they are used by natural enemies to locate herbivores attacking the plant and thus can function as an indirect defence for the plant (Dicke and Baldwin, 2010). Increased parasitization and predation rates of the herbivores can reduce plant damage and increase reproductive output (Huang et al., 2008; Rasmann et al., 2005).

This study shows that *de novo* produced compounds induced in cotton by herbivore feeding can play an important role in reducing further herbivore attack, and it may be beneficial for herbivores to respond to these compounds when searching for suitable host plants.

The *de novo* produced compounds can be used by herbivores as reliable cues to evaluate food quality for their offspring, as well as the risk for exploitative competition and parasitization.

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AUTHOR CONTRIBUTIONS

P.A. provided the initial concept for the experiments; A.Z., M.B., M.M.S., B.S.H., P.W. and P.A. designed the experiments; A.Z. and M.M.S. performed the experiments; A.Z., M.M.S., M.B. and P.A. analysed the data; and A.Z., M.B., M.M.S. and P.A. wrote the paper.

COMPETING INTERESTS

No competing interests declared.

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