

# Is quality more important than quantity? Insect behavioural responses to changes in a volatile blend after stemborer oviposition on an African grass

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Plants subjected to insect attack usually increase volatile emission which attracts natural enemies and repels further herbivore colonization. Less is known about the capacity of herbivores to suppress volatiles and the multitrophic consequences thereof. In our study, the African forage grass, *Brachiaria brizantha*, was exposed to ovipositing spotted stemborer, *Chilo partellus*, moths. A marked reduction in emission of the main volatile, (Z)-3-hexenyl acetate (Z3HA), occurred following oviposition but the ratio of certain other minor component volatiles to Z3HA was increased. While further herbivore colonization was reduced on plants after oviposition, the new volatile profile caused increased attraction of an adapted parasitoid, *Cotesia sesamiae*. Our results show that insect responses are dependent on the quality of volatile emission rather than merely the quantity in this multitrophic interaction.

**Keywords:** volatile emission; ratios; plant–insect interactions; multitrophic interactions

## 1. INTRODUCTION

Plants have evolved sophisticated defence mechanisms against attacking organisms ranging from direct antibiotic defences to more subtle indirect defences involving attraction of natural enemies of herbivores. Simultaneously, natural enemies of herbivores have evolved chemosensory systems that are fine-tuned for recognition of plants on which their prey is present.

Typically, insect attack leads to an increase in emission of plant volatile compounds that appear to function as a ‘cry for help’ to attract natural enemies in tritrophic interactions (Takabayashi & Dicke 1996; de Moraes *et al.* 1998; Du *et al.* 1998; Turlings & Ton 2006; Heil 2008) and repel further colonization by the herbivore itself (de Moraes *et al.* 2001; Kessler & Baldwin 2001). Recently, it has been observed that plants increase volatile emission even at the earliest stage of herbivore attack, namely oviposition (Colazza *et al.* 2004; Hilker & Meiners 2006). This reduces further colonization by the herbivore itself and

activates indirect defence, whereby natural enemies are attracted even before the larva has emerged. This ensures that biological control occurs early on and before larvae damage the plant. While many studies report that herbivore-induced plant volatiles are released in larger quantities following herbivore attack, less is known of the capacity for herbivores to suppress volatiles and the multitrophic consequences thereof. If some volatiles are suppressed while others are induced, this would cause a qualitative change in the odour profile which could be important if the ratio of volatiles emitted by a plant is used in host recognition by insects (Bruce *et al.* 2005).

Here, we investigate the chemical ecology of multitrophic interactions between an African grass used in subsistence farming systems and the stemborers that damage it, which are also important pests of maize. Two questions are addressed: (i) is volatile emission altered by oviposition of the herbivore? and (ii) are multitrophic interactions with a Braconid parasitoid affected by changes in volatile signals?

## 2. MATERIAL AND METHODS

Signal grass, *Brachiaria brizantha* (A.Rich.) Stapf (Poaceae), an important forage crop in Africa, was grown from root splits in pots in a screen house (natural light conditions, approx. L 12 : D 12 h) at ICIPE in Western Kenya. Spotted stemborer, *Chilo partellus* Swinhoe (Lepidoptera: Crambidae), moths were reared from larvae collected from the wild, principally sorghum fields, and fed on a natural diet of sorghum cv. Serena. *Cotesia sesamiae* Cameron (Hymenoptera: Braconidae wasps, larval parasitoids of *C. partellus*) were reared as described by Overholt *et al.* (1994).

Treated plants were caged with five gravid *C. partellus* overnight the night before headspace sampling while control plants were kept in identical conditions in empty cages. Collections began in the last 2 h of photophase the following day. Volatile compounds were collected for 48 h periods from intact plants using standard methods (Agelopoulos *et al.* 1999). Foliage (one plant per collection; mean weight = 20.6 g) was enclosed in polyethyleneterephthalate bags (volume, 3.2 l). Charcoal filtered air was pumped in and air was drawn out at 400 ml min<sup>-1</sup> through Porapak Q (0.05 g) filters.

Collected volatiles were eluted from Porapak Q with diethyl ether and analysed on a capillary gas chromatography (GC) column (50 m × 0.32 mm ID, HP-1) directly coupled to a mass spectrometer (Autospec Ultima, Micromass, UK). Ionization was by electron impact at 70 eV, 250°C. Oven temperature was maintained at 30°C for 5 min, then programmed at 5°C min<sup>-1</sup> to 250°C. Authentic (Z)-3-hexenyl acetate (Z3HA) (99% purity, Avocado Research Chemicals Ltd., Lancs, UK) was used as an external standard to calibrate GC peak area and quantify the amount present in each headspace sample.

Electroantennogram (EAG) recordings were made from female *C. sesamiae* and replicated five times. An antenna was excised and suspended between two Ag–AgCl glass electrodes filled with Ringer saline solution. Responses were measured in millivolt deflections and signals passed through a high impedance amplifier (UN-06, Syntech, The Netherlands). The coupled GC-electrophysiology system, in which effluent from a GC column is simultaneously delivered to the antennal preparation and a GC detector, has been described (Wadhams 1990). Separation of volatiles was achieved using an Agilent 6890 GC fitted with a non-polar column (HP-1, 50 m × 0.32 mm ID × 0.52 µm film thickness). Oven temperature was maintained at 40°C for 2 min, then programmed at 5°C min<sup>-1</sup> to 100°C and then at 10°C min<sup>-1</sup> to 250°C. Simultaneous records of the EAG and flame ionisation detector responses were obtained with commercial software (Syntech, The Netherlands).

A Perspex four-arm olfactometer (Petterson 1970) was used with air drawn through the four arms towards the centre at 350 ml min<sup>-1</sup>. Mated female *C. sesamiae* wasps, without previous exposure to plants or prey, were transferred individually into the central chamber. Time spent in each arm was recorded using OLFA (Udine, Italy) software over a 10 min period. Aliquots of 10 µl of headspace samples (dose similar to that emitted by 12 plants over 10 min) were applied to a filter paper strip inserted into an inlet port at the entrance of an arm. A choice test was carried out: one arm had a headspace sample from plants with oviposition while the

Table 1. Emission rates of volatiles from *B. brizantha* plants without and with *C. partellus* eggs.

	mean emission (ng plant <sup>-1</sup> h <sup>-1</sup> ) ( $\pm$ s.e.)		d.f.	n	t	p
	without eggs	with eggs				
6-methyl-5-hepten-2-one	5.75 ( $\pm$ 0.67)	5.01 ( $\pm$ 0.99)	14	8	0.62	0.55
(Z)-3-hexenyl acetate	202.25 ( $\pm$ 41.95)	68.28 ( $\pm$ 14.06)	8.5	8	3.03	0.015
(E)-ocimene	1.97 ( $\pm$ 0.33)	2.44 ( $\pm$ 0.96)	8.6	8	-0.46	0.66
(S)-linalool	5.73 ( $\pm$ 1.01)	6.22 ( $\pm$ 1.55)	14	8	-0.26	0.80
DMNT	12.43 ( $\pm$ 2.73)	9.55 ( $\pm$ 3.33)	14	8	0.67	0.52
methyl salicylate	1.73 ( $\pm$ 0.24)	2.87 ( $\pm$ 1.37)	7.4	8	-0.82	0.44
(E)-caryophyllene	2.58 ( $\pm$ 0.97)	1.87 ( $\pm$ 0.55)	14	8	0.64	0.53
TMTT	2.14 ( $\pm$ 0.83)	3.34 ( $\pm$ 1.02)	14	8	-0.92	0.37
total other	32.33 ( $\pm$ 4.67)	31.30 ( $\pm$ 7.84)	14	8	0.11	0.91
ratio (Z3HA : other)	6.78 ( $\pm$ 1.46)	3.01 ( $\pm$ 0.87)	14	8	2.23	0.04

opposite arm had a sample from clean plants. The remaining two arms were blank controls (solvent only).

In an oviposition bioassay, two four-week-old potted plants, one clean (no prior oviposition) and one with eggs (caged with five gravid female *C. partellus* moths the night before), were placed in a cage (80 × 40 × 40 cm) covered by a fine wire mesh netting. The following night, another five gravid female *C. partellus* moths were introduced into each cage and allowed to oviposit overnight. Plants were removed and the number of fresh eggs on each plant counted under a light microscope at ×6.5 magnification. Fresh eggs were distinguished by a difference in colour.

Data on oviposition preference were analysed with a two-sample *t*-test and mean times spent in olfactometer arms were compared by one-way analysis of variance (ANOVA) after confirming that data were normally distributed. Means were separated using Tukey's studentized range test. All analyses were performed using SAS (SAS Institute 2001).

### 3. RESULTS

#### (a) Volatile analysis

The main volatile emitted by uninfested *B. brizantha* was the green leaf volatile Z3HA. However, following oviposition by *C. partellus*, there was a marked reduction in its emission (mean reduction of 67%; table 1). Plants without eggs released a mean of 202 ng plant<sup>-1</sup> h<sup>-1</sup> Z3HA whereas plants with eggs released 68 ng plant<sup>-1</sup> h<sup>-1</sup>. Minor components in samples from plants not exposed to oviposition formed a greater proportion of the total volatile emission in exposed plants (table 1).

#### (b) Electrophysiological responses of parasitoids

GC-linked electrophysiological analyses, with parasitoid antennae and a headspace sample of *B. brizantha* exposed to *C. partellus* oviposition, revealed that *C. sesamiae* was responsive to Z3HA and six other compounds: 6-methyl-5-hepten-2-one, (E)-ocimene, (S)-linalool, (3E)-4,8-dimethyl-1,3,7-nonatriene (DMNT), (E)-caryophyllene and (3E,7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) (figure 1).

#### (c) Behavioural responses of parasitoids

There were significant differences in response by *C. sesamiae* to the odours tested in dual-choice olfactometer bioassays ( $p = 0.0028$ ,  $f = 6.53$ ,  $n = 20$ , d.f. = 2). The volatile blend emitted from plants exposed to *C. partellus* oviposition was preferred

to that of volatiles collected from plants without oviposition (Tukey's HSD:  $p < 0.05$ ; figure 2).

#### (d) Oviposition bioassay

In oviposition bioassays, *B. brizantha* plants with eggs (mean number of eggs already present = 126.8) were less preferred for subsequent oviposition by *C. partellus*. A mean of 126.3 (s.e. =  $\pm 9.51$ ) eggs were laid on plants without eggs, while a mean of 81.2 (s.e. =  $\pm 9.18$ ) new eggs were laid on plants with eggs which was significantly less ( $p = 0.002$ ,  $t = 3.41$ ,  $n = 10$ , d.f. = 18).

### 4. DISCUSSION

Our study demonstrates that qualitative as well as quantitative changes in plant volatile emission following herbivory can influence interactions with associated insects. The volatile blend emitted by the African forage grass, *B. brizantha*, was altered following oviposition by the stemborer pest, *C. partellus*, with the main change being suppression of Z3HA emission. This was unusual because insect attack usually induces increases not decreases in plant volatile emission (Heil 2008 and references therein). However, the ratio of other compounds relative to Z3HA was increased in plants exposed to *C. partellus* oviposition (table 1). Despite the reduction in total amount of volatile emission, volatiles of plants exposed to oviposition were found to be more attractive to an indigenous and highly adapted natural enemy of the herbivore, a larval parasitic wasp, *C. sesamiae*, than those of plants without oviposition.

Electrophysiological analyses revealed that *C. sesamiae* is sensitive to the minor compounds 6-methyl-5-hepten-2-one, (E)-ocimene, (E)-caryophyllene, TMTT and DMNT which have been previously reported as having a function in tritrophic interactions (Turlings & Tumlinson 1992; Turlings *et al.* 1995; Khan *et al.* 1997; de Moraes *et al.* 1998; Du *et al.* 1998). Thus, while production of the major semiochemical was suppressed, minor cues were enhanced thereby allowing insects still to recognize the colonized plant. Insect responses to ratios of volatiles are important in chemical ecology, and perception of blends in particular ratios has emergent properties not explained by the constituent

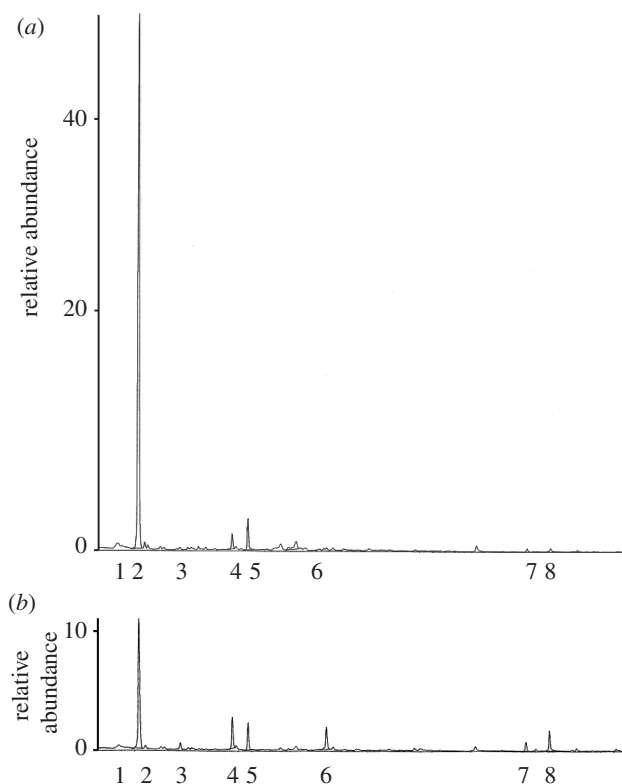


Figure 1. GC analysis of *B. brizantha* volatiles: (a) volatile profile of a plant not exposed to *C. partellus* oviposition, (b) volatile profile exposed to oviposition by *C. partellus*. Represented are 1, 6-methyl-5-hepten-2-one; 2, Z3HA; 3, (*E*)-ocimene; 4, (*S*)-linalool; 5, (*3E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT); 6, methyl salicylate; 7, (*E*)-caryophyllene; 8, (*3E,7E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT).

compounds (Bruce *et al.* 2005). In this case, the ratio of other compounds to Z3HA appears to be an important component of the volatile signal perceived by insects associated with *B. brizantha*.

Furthermore, plants with eggs were less preferred for subsequent oviposition by the herbivore itself, suggesting that the volatile changes could act as an epideictic signal to avoid intraspecific crowding that would occur if eggs were repeatedly deposited on the same plant. Visual cues from the eggs themselves or non-volatile contact chemoreception of egg chemicals could also have played a role in the oviposition bioassay, but these cues were not present in the olfactometer bioassay with the parasitoid.

There is an urgent need in most of rural Africa, where farmers cannot afford chemical pesticides to protect their crops, for crop varieties which can naturally defend themselves against herbivory by exploiting plant–natural enemy communication. The current findings could provide insights into selecting and breeding maize varieties with better potential for induced early defence against pests such as *C. partellus*.

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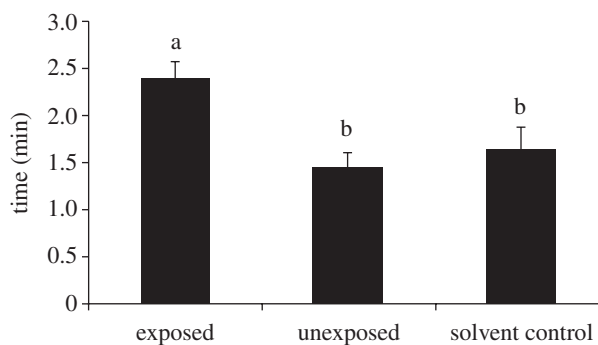


Figure 2. Olfactometer responses of *C. sesamiae* female parasitoid wasps to volatiles from *B. brizantha* plants with and without *C. partellus* oviposition ( $n = 20$ ). Time spent (mean  $\pm$  1 s.e.) in different olfactometer areas are shown.

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