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Identification of Host Kairomones from Maize, *Zea mays*, for the Maize Weevil, *Sitophilus zeamais*

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Abstract The maize weevil, *Sitophilus zeamais*, is an economically important pest of stored grains in tropical and subtropical regions of the world. The behavioral responses of adult *S. zeamais* to volatile organic compounds (VOCs) from maize seeds, *Zea mays*, were studied to identify semiochemicals used in host location and provide potential tools for managing this pest. VOCs released directly from crude seed extracts, vacuum distilled extracts, hexane and diethyl ether fractions from silica gel chromatography of the vacuum distillates, air entrainment samples, and identified volatile compounds were assayed using a Perspex four-arm olfactometer. Weevils spent significantly more time, and made a higher number of visits, to the region of the olfactometer where *Z. mays* volatiles were present than in control regions comprising solvent only. When white and yellow *Z. mays* VOCs were compared in a choice test, the mean time spent in the two olfactometer treatment arms was significantly greater than the mean time spent in the control arms. However, weevils did not show any preference for either of the two treatments, thus demonstrating that both varieties of maize have similar activity. Gas chromatography (GC), coupled gas chromatography-mass spectrometry (GC-MS), GC peak enhancement and electroantennography (EAG) identified hexanal, (*E*)-2-heptenal, and octanal as biologically active compounds in air entrainment samples and diethyl ether fractions of vacuum distillates. A 3-component synthetic blend of the identified compounds was significantly attractive to both sexes of the weevil. These host kairomones could be deployed in semiochemical based monitoring and management of *S. zeamais* in the tropics.

Keywords *Zea mays* · *Sitophilus zeamais* · Air entrainment · Headspace collection · Volatile organic compounds · Olfactometry · EAG · Hexanal · (*E*)-2-Heptenal · Octanal · Coleoptera · Curculionidae

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Introduction

The maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae), is an important economic pest of stored grains in tropical and sub-tropical regions of the world. It attacks various stored foodstuffs including maize, wheat, oats, barley, rye, and dried cassava roots, as well as processed food such as macaroni, noodles, biscuits, and hardened cake. Post-harvest crop losses due to storage pests such as *S. zeamais* pose major problems to food security in Africa. In Nigeria, the loss of maize grains during storage due to insect pests such as *S. zeamais* has long been a serious problem to farmers. Inputs in the form of human effort and finances invested in the production of the crop are wasted. *Sitophilus zeamais* infest ripening standing crops immediately prior to harvest and in storage, causing damage by boring into the grains and eating the inner part, which reduces maize weight and quality in terms of consumption and germination (Kossou and Bosque-Berez, 1998; Adda et al., 2002; Ukeh and Udo, 2008). The activity of *S. zeamais* larvae and adults may be associated with weight losses by direct damage and physical contamination, lowering the nutritional and economic value of the crop, and the presence of allergens (Arlian, 2002; Ukeh et al., 2010) or toxinogenic fungi (Hubert et al., 2002) in the infested stored grain. *S. zeamais* infestation also results in significant reduction in the viability of maize grains (Rees, 2004).

Fumigation of large scale storage granaries by the application of synthetic chemicals such as methyl bromide and aluminium phosphate has been used to control storage pest species (Lale, 2002; Sousa et al., 2008). However, regulatory restrictions as a result of adverse effects on non-target organisms, increasing consumer demand for food safety (Germinara et al., 2008), loss of efficacy, development of pest resistance, human and ecotoxicity (Lorini et al., 2007; Sousa et al., 2009; Pimentel et al., 2009), financial and technical limitations at the critical periods of need (Ukeh et al., 2012), all make the control of stored product pests difficult. Furthermore, since larval and pupal instars develop within the maize grains, any sustainable control strategy demands the timely and accurate monitoring of the prevalence of adult weevils.

Phytophagous insects use plant volatile cues to locate resources appropriate for their different requirements, including their preferred host plants for food and oviposition sites (Visser, 1986; Pickett et al., 1998; Van den Berg et al., 2008). This complex process is mediated by the synthesis of numerous sensory inputs within the insect central nervous system, including olfactory or gustatory semiochemical cues, as well as physical and visual information such as plant texture, color and shape (Bruce et al., 2005). For instance, the stemborers *Chilo partellus* and *Busseola fusca* respond to volatiles emitted by *Zea mays* and *Sorghum bicolor* (cultivated host plants), and *Pennisetum purpureum* and *Hyparrhenia tamba* (wild grass hosts), indicating host location kairomones used by the stemborers for feeding and oviposition under field conditions (Birkett et al., 2006). The coffee berry borer, *Hypothenemus hampei*, has been reported to exhibit behavioral responses to ripe and dry fruit volatiles of its host plant, *Coffee arabica* (Mendesil et al., 2009). The utilization of semiochemicals for the control of stored-product pests has stimulated global interest. Germinara et al. (2008) reported that *S. granarius* adults have the ability to respond behaviorally to a wide range of cereal volatiles. White maize is locally adapted and is a widely cultivated maize variety in Nigeria, but in recent years, yellow maize containing carotene also has been cultivated because of additional health benefits. Recently, Ukeh et al. (2010) reported the behavioral responses of *S. zeamais* to volatiles from seeds of winter wheat, *Triticum aestivum*, and Nigerian white and yellow maize in olfactometer studies. An improved understanding of *S. zeamais* olfaction could facilitate the development of novel crop protection strategies based on semiochemicals to reduce weevil populations in and around storage granaries. Previous laboratory based studies showed that seeds of alligator pepper, *Aframomum melegueta*, and ginger rhizomes, *Zingiber officinale*, were repellent to adult *S. zeamais*, with the observed repellency being accounted for by the presence of volatile organic compounds (VOCs) (Ukeh et al., 2009). Oxidation of lipids present in seed oils leads to the generation of VOCs (Belitz et al., 2009). To test this hypothesis, the main objective of this study was to identify the VOCs released by grains of maize, *Z. mays*, that are attractive to *S. zeamais*. Identification of the attractant cues would provide the underpinning science required to establish the combined use of a maize trap seed, and *A. melegueta* and/or *Z. officinale*, in a push-pull strategy for harvested maize protection at the smallholder farm level in sub-Saharan Africa (Khan et al., 2010).

Methods and Materials

Insect Culture and Plant Materials A starter population of *S. zeamais* was obtained from the Food and Environment Research Agency, Sand Hutton, York (UK) on January 13, 2011, and cultured on Nigerian white and yellow maize grains at Rothamsted Research, Harpenden (UK). Cultures were maintained in a standing incubator running at constant temperature of 25 °C and 65 % relative humidity under a 12:12 L:D photoperiod (Vindon Scientific Ltd. Rochdale, UK). Untreated Nigerian white and yellow maize, *Z. mays*, seeds were purchased from the Maize and Cassava Cooperative society in Obudu Local Government Area of Cross River State (situated within latitude 6°39′ and 43°38′ North and longitude 9°09′ and 10°01′ East, elevation of 194.16 m) in southern Nigeria. Seeds were sun-dried to a moisture content of about 11–13 % before transportation, and preserved at −5 °C at Rothamsted Research until needed.

White and Yellow Maize Seed Extraction One hundred and fifty grams (150 g) of white and yellow *Z. mays* seeds were frozen with liquid nitrogen, and ground using a laboratory mortar and pestle. Ground materials were extracted using freshly distilled diethyl ether (200 ml each) for 24 hr at ambient temperature, with additional stirring using a magnetic stir bar. The contents were filtered (gravity filtration), and the residue was re-extracted for another 24 hr with diethyl ether (200 ml). Combined extracts were evaporated under reduced pressure, then under a gentle stream of nitrogen to a volume of ca. 10 ml; ca. 5 ml of extract were transferred into a 100 ml round-bottomed flask connected to vacuum distillation apparatus equipped with a high vacuum pump, and distilled at ambient temperature under a vacuum of <0.1 mm Hg for 24 hr, following the methods of Pickett and Griffiths (1980) and Ukeh et al. (2009). The distillates were transferred to glass vials using a long-drawn Pasteur pipette, and stored at −20 °C until needed for bioassays, liquid chromatography or chemical analysis.

Liquid Chromatography White and yellow *Z. mays* vacuum distillates (1 ml) were concentrated under a stream of

nitrogen to dryness, and immediately redissolved in distilled hexane (50 μl). The reconstituted extracts were subjected to liquid chromatography through silica gel (40–60 mesh) following the procedures described in Birkett et al. (2008). Distilled hexane and diethyl ether were used sequentially as eluants to obtain a hexane fraction containing non-polar compounds and a diethyl ether fraction containing polar compounds, respectively. Fractions were concentrated to their original 1 ml volume prior to use in behavioral assays.

Air Entrainment This employed dynamic headspace collection, carried out following the procedure described in Webster et al. (2008). All equipment used for the collection of volatile organic compounds (VOCs) was washed with an aqueous solution of Teepol detergent (Herts County Supplies, Hertfordshire, UK), rinsed with acetone and distilled water, and baked overnight in an oven running at 160 °C. Porapak Q tubes were eluted with redistilled diethyl ether and heated at 132 °C for 2 hr to remove contaminants. One hundred grams (100 g) each of the untreated Nigerian white and yellow *Z. mays* seeds were placed in 3-necked round-bottomed flasks (250 ml). Charcoal filtered air was pumped at 500 $ml\,min^{-1}$ through an inlet and drawn out at 500 $ml\,min^{-1}$ through a 5 mm diam glass tube containing 50 mg Porapak Q (Alltech Associates, Camforth, Lancashire, UK). The connections were made airtight using PTFE tubing (Alltech Associates, Lancashire, UK) with Swagelock fittings (North London Valve, UK) and sealed with PTFE tape (Gibbs and Dandy, Luton, UK). Seeds were entrained for 7 day, and the Porapak Q filter was eluted with redistilled diethyl ether (0.5 ml) to provide a solution containing the isolated VOCs. For chemical analysis, entrained samples were concentrated under a gentle stream of nitrogen to ca. 50 μl and stored in tightly capped microvials at −20 °C until needed.

Olfactometer Assays Behavioral assays with insects were carried out using a Perspex four-arm olfactometer (Pettersson, 1970) to determine the responses of <3 day old adult *S. zeamais* to crude *Z. mays* seed extracts, vacuum distilled *Z. mays* extracts, distilled hexane and diethyl ether *Z. mays* fractions, and air entrainment samples. Detailed construction of the olfactometer and procedures of bioassays have been described (Webster et al., 2008; Ukeh et al., 2010). In the single choice tests, one arm of the olfactometer contained the treatment (odor source) while the remaining three arms served as controls. The stimuli included 10 μl *Z. mays* whole extracts, 10 μl *Z. mays* vacuum distillates, 10 μl vacuum distilled hexane or diethyl ether fractions, 10 μl air entrainment sample, 10 μl synthetic compound tested singly, and 10 μl of a 3-component synthetic blend with a concentration of 10 ng/μl of each compound, loaded on a filter paper disc in the test arm, vs. 10 μl solvent impregnated on filter discs as controls. With the aid of a Nikon binocular microscope, insects were sexed following the methods of Halstead (1963) and Haines (1991) according to the dimorphic rostral characteristics in which males have a distinctly shorter, wider, and rougher rostrum than females. Weevils were starved for 24 hr prior to bioassays and a single weevil was introduced through a hole in the top of the olfactometer with a fine paintbrush. Air was drawn through the central hole at a rate of 400 $ml\,min^{-1}$ and subsequently expelled from the room. Each weevil was observed for 16 min, and the olfactometer was rotated 90° every 2 min in a clockwise direction to control for any directional bias. Test insects, olfactometers and odor samples or stimuli were changed after every replication. All experiments were replicated 12 times, and data on time spent and number of entries made by *S. zeamais* to test and control arms were recorded with Olfa computer software (F. Nazzi, Udine, Italy).

Data Analysis Data on the mean time spent in, and number of entries (visits) into, treated and control arms of the olfactometer were the parameters chosen for assessment of the differences between odor sources. The null hypothesis of equal time spent in, and number of visits to, each olfactometer arm was tested using analysis of variance (ANOVA) after checking that the data were normally distributed. The responses of weevils in EAG recordings to control and test solutions were also analysed using ANOVA. Means were compared using Tukey's 95 % simultaneous confidence intervals (Genstat 13).

Gas Chromatography (GC) Analysis Diethyl ether fractions of vacuum distillates of *Z. mays*, and air entrainment samples, were analyzed on an Agilent 6890 GC (Agilent Technologies, UK) equipped with a cold on-column injector, a flame ionization detector (FID), a non-polar HP-1 bonded-phase fused silica capillary column (50×0.32 mm i.d., film thickness 0.52 μm), and a polar DB-WAX column (30 m×0.32 mm i.d. 0.52 μm film thickness). Oven temperature was maintained at 30 °C for 1 min, and programmed at 5 $°C\,min^{-1}$ to 150 °C and held for 0.1 min, then 10 $°C\,min^{-1}$ to a final hold at 230 °C for 50 min. Hydrogen was the carrier gas. Results were obtained with an enhanced integrator (HP Chemstation).

Coupled Gas Chromatography-Mass Spectrometry (GC-MS) Behaviorally active white and yellow *Z. mays* diethyl ether fractions, and air entrainment samples, were analyzed on a capillary GC column (HP-1, 50 m, 0.32 mm i.d., 0.52 μm film thickness) directly coupled to a mass spectrometer (VG Autospec Ultima). Ionization was made by electron impact at 70 eV, 250 °C. Oven temperature was maintained at 30 °C for 5 min and then programmed at 5 °C min^{-1} to 250 °C. Tentative GC-MS identifications were made by comparison of spectra with mass spectral databases

(NIST, 2005), and confirmed by peak enhancement on GC using two columns of differing polarity (DB-1 and DB-WAX) with authentic compounds.

Synthetic Chemicals Hexanal (>99 % purity), nonanal (>95 %), (*E*)-2-heptenal (>99 %), and decanal (>99 %) were purchased from Sigma-Aldrich. Octanal (>98 %) was purchased from Avocado.

Electroantennography Electroantennogram (EAG) recordings were made using Ag-AgCl glass electrodes filled with a saline solution as described in Maddrell (1969) but without glucose. An antenna was excised and suspended between the two electrodes. The tip of the terminal process of the antenna was scraped with a fine scalpel to ensure a good contact. The signals were passed through a high impedance amplifier (UN-06 Syntech, The Netherlands) and analyzed using a customized software package (Syntech). The stimulus delivery system utilized a filter paper strip in a disposable Pasteur pipette cartridge (Wadhams et al., 1982). The stimulus (2 sec duration) was delivered into a purified airstream (1 l/min) flowing continuously over the preparation. Samples (10 µl) of the standard solutions (1 mg/ml in hexane) of each test compound [hexanal, nonanal, (*E*)-2-heptenal, decanal, or octanal] were applied to filter paper strips, and the solvent was allowed to evaporate for 30 sec before the strip was placed in the cartridge. The control stimulus was hexane (10 µl) and each treatment was replicated ten times. Fresh cartridges were prepared immediately prior to each stimulation. Data were normalized as a percentage response compared to the control.

Results

Behavioral Responses to White and Yellow Maize Extracts In preliminary tests, there were no significant differences in the time spent by male and female *S. zeamais* in the four arms of the olfactometer when diethyl ether was the control treatment (Table 1). With diethyl ether extracts of white and yellow *Z. mays*, male and female weevils spent significantly more time in the region of the olfactometer where the maize extracts were present (Table 1). Male and female *S. zeamais* also made a higher number of visits to the arm containing the white or yellow maize extracts (Table 2). When white and yellow *Z. mays* extracts were assayed in a dual choice test, the total time spent by adult *S. zeamais* in the two treatment arms was significantly greater than the total time spent in the two control arms ($P<0.001$) (Table 3). Male and female weevils reared on white or yellow *Z. mays* showed a significant preference for white or yellow *Z. mays* in time spent, compared to control arms (Table 3).

Behavioral Responses to Maize Vacuum Distillates, Silica Gel Chromatography Using white or yellow *Z. mays* vacuum distillates as the stimulus source, male and female *S. zeamais* spent significantly more time in the treatment arm than in the controls (Table 4). For hexane and diethyl ether fractions of white and yellow *Z. mays* vacuum distillates, prepared by silica gel chromatography, there was no significant response of male or female *S. zeamais* to hexane fractions (Table 4) compared to control arms. However, the diethyl ether fractions of the white and yellow *Z. mays* distillates were significantly attractive to male and female weevils compared to control arms (Table 4).

Behavioral Responses to Maize Air Entrainment Volatiles Male and female *S. zeamais* spent significantly more time in the region of the olfactometer where the white and yellow *Z. mays* entrainment samples were presented (Table 5).

Chemical Analysis and Electrophysiology Coupled GC-MS analysis of the diethyl ether fractions of the yellow and white *Z. mays* vacuum distillates, and air entrainment samples, led to the identification of several aliphatic aldehydes (Fig. 1). (*E*)-2-Heptenal, hexanal, and octanal showed significant EAG responses at the dose tested (Fig. 2), but nonanal and decanal, which were detected in trace amount in volatile samples, showed no significant EAG activity (Fig. 2).

Behavioral Responses to Single Compounds and a Synthetic Blend Male and female *S. zeamais* showed no significant

Table 1 Mean time spent (min ± SE) by adult *Sitophilus zeamais* in response to *Zea mays* seed extracts in a 4-arm olfactometer

Treatments	Mean time spent*	
	Males	Females
Control 1	3.84±0.64a	3.83±0.06a
Control 2	3.69±0.10a	3.73±0.06a
Control 3	3.78±0.09a	3.73±0.07a
Control 4	3.85±0.08a	3.77±0.06a
White *Z. mays* extract	7.71±0.81a	6.43±0.57a
Control 1	2.16±0.29b	2.65±0.23b
Control 2	2.55±0.37b	2.91±0.25b
Control 3	2.38±0.29b	2.91±0.27b
Yellow *Z. mays* extract	7.15±0.64a	6.94±0.45a
Control 1	2.61±0.29b	2.54±0.11b
Control 2	2.43±0.18b	2.57±0.24b
Control 3	2.49±0.22b	2.51±0.17b

*Within a block of four treatments, means followed by a different letter are significantly different at $P=0.05$. $N=12$

Table 2 Mean number of entries (± SE) by adult *Sitophilus zeamais* in response to *Zea mays* seed extracts in a 4-arm olfactometer

Treatments	Mean number of entries*	
	Males	Females
Control 1	4.67±0.22a	4.42±0.29a
Control 2	5.08±0.26a	4.58±0.23a
Control 3	5.08±0.34a	4.42±0.33a
Control 4	4.33±0.45a	4.17±0.41a
White *Z. mays* extract	9.67±1.39a	8.25±0.97a
Control 1	4.33±0.68b	4.67±0.71b
Control 2	4.00±0.49b	5.00±0.66b
Control 3	4.67±0.48b	5.25±0.72b
Yellow *Z. mays* extract	11.08±1.32a	9.92±0.75a
Control 1	5.33±0.53b	5.42±0.47b
Control 2	5.42±0.59b	6.08±0.58b
Control 3	6.00±0.69b	5.50±0.73b

*Within a block of four treatments, means followed by a different letter are significantly different at P=0.05. N=12

responses to hexanal, (*E*)-2-heptenal or octanal when tested as single compounds (Table 6). However, male and female *S. zeamais* spent significantly more time in the region of the olfactometer where a 3-component synthetic blend was used as an odor source compared to the control (Table 6).

Table 3 Mean time spent (min ± SE) by adult *Sitophilus zeamais*, cultured on different maize varieties, in response to white and yellow *Zea mays* seed extracts when both odor sources were presented together in a 4-arm olfactometer

Treatments	Mean time spent*	
	Males	Females
A. Reared on white *Z. mays*		
White *Z. mays* extract	6.41±0.70a	6.13±0.57a
Yellow *Z. mays* extract	5.42±0.78a	5.12±0.46a
Control 1	1.55±0.28b	1.91±0.45b
Control 2	1.55±0.26b	1.99±0.38b
B. Reared on yellow *Z. mays*		
White *Z. mays* extract	5.32±0.41a	5.26±0.32a
Yellow *Z. mays* extract	5.22±0.25a	5.54±0.55a
Control 1	2.38±0.27b	2.21±0.33b
Control 2	2.15±0.30b	2.14±0.38b

*Within a block of four treatments, means followed by a different letter are significantly different at P=0.05. N=12

Table 4 Mean time spent (min ± SE) by adult *Sitophilus zeamais* in response to *Zea mays* vacuum distillates, and hexane and diethyl ether fractions, in a 4-arm olfactometer

Treatments	Mean time spent*	
	Males	Females
White *Z. mays* vacuum distillate	5.42±0.39a	5.71±0.22a
Control 1	3.30±0.32b	2.78±0.27b
Control 2	3.12±0.21b	3.35±0.15b
Control 3	3.02±0.16b	3.22±0.12b
Yellow *Z. mays* vacuum distillate	5.33±0.38a	5.66±0.59a
Control 1	3.33±0.22b	3.25±0.37b
Control 2	3.17±0.22b	3.02±0.29b
Control 3	3.16±0.22b	2.88±0.29b
White *Z. mays* hexane fraction	3.90±0.47a	3.44±0.22a
Control 1	3.63±0.41a	3.87±0.47a
Control 2	3.55±0.41a	3.63±0.41a
Control 3	3.79±0.28a	4.14±0.41a
White *Z. mays* diethyl ether fraction	5.43±0.51a	5.09±0.58a
Control 1	2.77±0.42b	3.69±0.33b
Control 2	3.11±0.36b	3.27±0.38b
Control 3	3.63±0.48b	3.11±0.32b
Yellow *Z. mays* hexane fraction	3.74±0.29a	3.60±0.39a
Control 1	4.02±0.32a	4.36±0.46a
Control 2	3.75±0.31a	3.73±0.25a
Control 3	3.70±0.28a	3.68±0.24a
Yellow *Z. mays* diethyl ether fraction	6.36±0.72a	5.44±0.59a
Control 1	3.13±0.42b	3.08±0.32b
Control 2	2.13±0.44b	3.13±0.32b
Control 3	2.62±0.36b	3.25±0.26b

*Within a block of four treatments, means followed by a different letter are significantly different at P=0.05. N=12

Discussion

In this study, volatiles collected from white and yellow *Z. mays* by either solvent extraction or air entrainment were significantly attractive to *S. zeamais*. The behavioral response of *S. zeamais* to host plant volatiles was independent of the sex of the weevil. This indicates that *S. zeamais*, like other phytophagous insects, uses plant volatiles during the search for food and oviposition sites. Plants produce a complicated variety of volatiles in varying amounts that influence ecological interactions with other plants and animals (Dudareva et al., 2004; Knudsen et al., 2006).

Table 5 Mean time spent (min ± SE) by adult *Sitophilus zeamais* in response to white and yellow *Zea mays* air entrainment volatiles in a 4-arm olfactometer

Treatments	Mean time spent*	
White *Z. mays* volatiles	4.97±0.23a	5.07±0.51a
Control 1	3.21±0.33b	2.93±0.36b
Control 2	3.14±0.30b	3.95±0.49b
Control 3	3.47±0.31b	2.98±0.48b
Yellow *Z. mays* volatiles	4.84±0.55a	5.22±0.72a
Control 1	3.31±0.31b	3.32±0.46b
Control 2	3.77±0.39b	3.50±0.47b
Control 3	3.22±0.29b	2.72±0.29b

*Within a block of four treatments, means followed by a different letter are significantly different at $P=0.05$. $N=12$

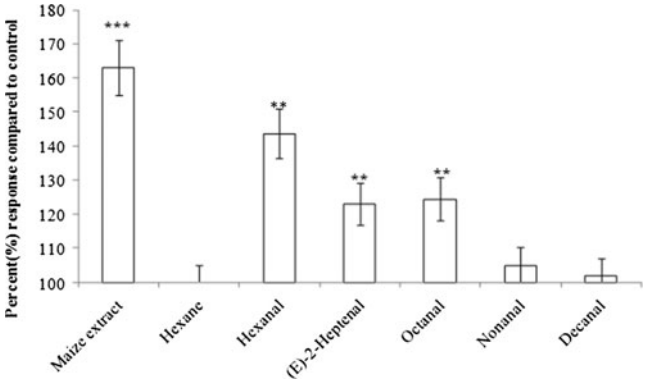

Fig. 2 Electroantennography (EAG) responses of adult *Sitophilus zeamais* to a maize extract and compounds (1 mg/ml) identified in the air entrainment sample of *Zea mays* seeds. Results expressed as a percentage (%) of responses to the solvent control. *Asterisks* indicate the activation thresholds; $N=10$

Phytophagous insects rely on these volatile cues in their search for host plants for food, mating, oviposition and in the avoidance of unsuitable hosts (Sole et al., 2010; Bruce and Pickett, 2011). *S. zeamais* was shown to be attracted to volatiles from seeds of winter wheat, *T. aestivum*, and white and yellow *Z. mays* in 4-arm olfactometer studies (Ukeh et al., 2010), but the host kairomones were not identified.

Data from this study showed that there was no significant difference in the attractiveness of volatile extracts collected from white or yellow *Z. mays*. GC and coupled GC-MS analysis of extracts revealed the presence of aliphatic aldehydes, of which hexanal, (*E*)-2-heptenal, and octanal were electrophysiologically active. These compounds, which were present in differing quantities and ratios between the two types of maize, comprise primary products of oxidation of lipids present in seed oils, including those found in maize (Belitz et al., 2009), and based on the electrophysiological data, it was hypothesised that they are a reliable olfactory cue for location of suitable food and oviposition sites for *S. zeamais*. However, in further behavior assays conducted with individual compounds, no significant attraction was observed. The equal attractiveness of white and yellow maize extracts, and the divergence of aldehyde quantities

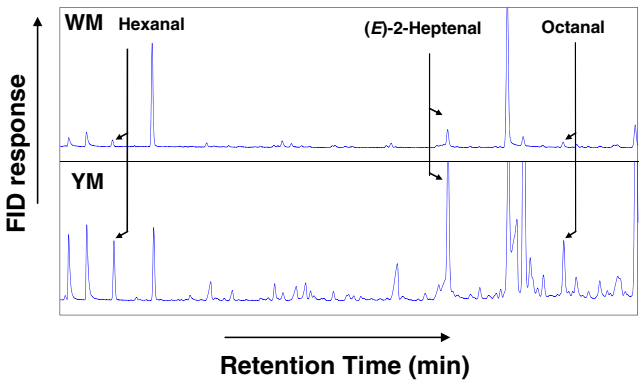

Fig. 1 Gas chromatography (GC) analysis of volatile organic compounds (VOCs) emitted by white (WM) and yellow (YM) *Zea mays* seeds, collected by air entrainment

Table 6 Mean time spent (min ± SE) by adult *Sitophilus zeamais* in response to hexanal, (*E*)-2-heptenal and octanal (each at 10 ng/ul concentration) and a synthetic blend of the compounds (1:1:1, 10 ng/μl) in a 4-arm olfactometer

Treatments	Mean time spent*	
	Males	Females
Hexanal	3.96±0.37a	4.73±0.69a
Control 1	4.11±0.29a	3.28±0.49a
Control 2	3.86±0.28a	4.36±0.81a
Control 3	2.99±0.44a	2.46±0.44a
(*E*)-2-Heptenal	3.54±0.29a	3.37±0.27a
Control 1	3.54±0.47a	3.80±0.29a
Control 2	4.18±0.39a	3.62±0.36a
Control 3	3.77±0.39a	4.30±0.41a
Octanal	4.23±0.33a	4.35±0.45a
Control 1	3.70±0.39a	3.74±0.32a
Control 2	3.46±0.28a	3.19±0.26a
Control 3	3.50±0.28a	3.96±0.29a
Synthetic blend	4.70±0.49a	4.82±0.32a
Control 1	3.46±0.22b	3.43±0.32b
Control 2	3.53±0.28b	3.38±0.19b
Control 3	3.07±0.28b	3.23±0.41b

*Within a block of four treatments, means followed by a different letter are significantly different at $P=0.05$. $N=12$

and ratios between extracts, determined that an artificial blend (1:1:1) of the aldehydes be tested, with the mixture being significantly more attractive to both sexes of *S. zeamais* than the solvent control. The implication of these findings is that *S. zeamais* shows stronger behavioral responses to combinations of volatiles than to single compounds. Similar studies have been demonstrated for alate black bean aphids, *Aphis fabae* (Webster et al., 2008), chestnut gall wasp, *Dryocosmus kuriphilus* (Germinara et al., 2011), and the West Indian fruit fly, *Anastrepha oblique* (Malo et al., 2012). In the study by Webster et al. (2008), it was shown that a synthetic blend of electrophysiologically active compounds from host plants was as attractive as a natural VOC blend, but when compounds were tested individually, they were shown to be significantly repellent. The results from this study confirmed that plant volatiles detected individually by *S. zeamais*, outside the context of the blend, could be perceived as unsuitable cues, but when they are combined together in a blend, they are perceived as an attractive host stimulus (Bruce and Pickett, 2011).

The results in this study show that a mixture of three components identified from *Z. mays* seed volatiles is attractive to *S. zeamais* and may be utilized by the weevil for habitat or host location. Since the efficacy of pheromone lures could be enhanced by combination with host odor cues (Landolt and Phillips, 1997), a blend of the identified kairomones deployed in baited traps with sticky bases, alone or in combination with pheromone lures, could be useful in monitoring *S. zeamais* in storage houses. Host plant volatiles could play a role in modifying the behavior of both male and female weevils, as direct attractants or oviposition stimulants, or as potential synergists for the male-produced aggregation pheromone. The use of attractants in traps significantly improves trap performance by increasing the chance of detecting even low-density pest infestations (Mahroof and Phillips, 2008). Furthermore, the results obtained in this study provide underpinning science for the deployment of trap seed as part of a 'push-pull' strategy for *S. zeamais* control in stored maize conditions, in conjunction with locally produced repellents (Ukeh et al., 2009, 2010, 2012), analogous to that used for control of stemborer moths in smallholder maize farms (Khan et al., 2010). Ongoing work is attempting to demonstrate the efficacy of these findings under controlled tropical grain storage conditions.

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