



Semiochemical modulation of host preference of *Callosobruchus maculatus* on legume seeds



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ABSTRACT

Host preference of *Callosobruchus maculatus* (F.) on seeds of three legume cultivars, Ife-brown and black-eyed cowpeas [*Vigna unguiculata* L. (Walp)], and soybean (*Glycine max* L.), was investigated. Mated female *C. maculatus* showed high (90–95%) attraction to the three legume cultivars in Y-tube bioassays. However, the weevils discriminated among the cultivars in four-choice tests and showed greater attraction to Ife-brown cowpea (50%) than to soybean (30%) and black-eyed cowpea (15%). Coupled gas chromatography-electroantennography (GC-EAD) and GC-MS analyses of the headspace volatile organic compounds (VOCs) emitted by the legume seeds identified 2-ethyl hexanol as the principal EAD active component. Emission of 2-ethyl hexanol was two-fold greater in Ife-brown cowpea ($\sim 0.54 \mu\text{g g}^{-1}$ seeds) compared with black-eyed cowpea ($\sim 0.23 \mu\text{g g}^{-1}$ seeds) and soybean ($\sim 0.21 \mu\text{g g}^{-1}$ seeds). Synthetic 2-ethyl hexanol attracted 68% of female *C. maculatus* at 0.01 μg dose in Y-tube bioassays. These results demonstrated that host preference in *C. maculatus* is odor-mediated, and identified 2-ethyl hexanol as a potential attractant for *C. maculatus*.

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1. Introduction

Cowpea [*Vigna unguiculata* L. (Walp.)], is an annual legume, widely grown in Africa, Latin America, Southeast Asia and the Southern United States (Abate et al., 2012). Cowpea is essential to the sustenance of millions of people in sub-tropical regions of Africa and Asia as the major protein-rich staple food which complements their cereal grains and starchy tuber crops-based diet (Duke, 1990). Insect pests have been a major challenge in production of cowpeas especially during storage. The cowpea weevil, *Callosobruchus maculatus* (F.) is a pest of many legumes especially *Vigna* spp. (Natural Resource Institute (NRI, 1996; Lale, 2002; Rees, 2004; Swella and Mushobozy, 2009). Annually, Africa loses 50–90% of stored cowpeas to *C. maculatus* (International Institute of Tropical Agriculture (IITA, 1989). This pest has traditionally been managed using multiple applications of broad-spectrum conventional insecticides (Lale, 2002; Rees, 2004). However, use of conventional

insecticides has various drawbacks including insecticide resistance and chemical residue in food (NRI, 1996).

Insects use semiochemicals to attack intruders, identify members of same colony, and find mates, food and oviposition sites (Phillips, 1997; Walgenbach et al., 1987; Sonenshine, 1985; Burkholder, 1990; Foster and Harris, 1997). The selection of an oviposition site by adult female insect is vital to the survival of her offspring because the larvae are destined to feed on the selected plant species (Rees, 2004; IITA, 1989). Therefore, female insects lay their eggs with great precision on the best available host plant (Awmack and Leather, 2002; Scheirs et al., 2003). The role of chemical cues in host location and acceptance in female insects makes them vital agents for selective insect pest control (Burkholder, 1990). The understanding and identification of the chemical cues in insects have been employed in developing semiochemical-based management strategies for some insect pests of field crops and stored grains (Phillips et al., 1993, 1996; Sharma and Fadamiro, 2003).

Bruchid species are known to exhibit specificity in the choice of legumes they attack (Rees, 2005). *C. maculatus* is primarily a pest of cowpea but has many alternative hosts among leguminous seeds. However, little is known about the mechanisms of host location

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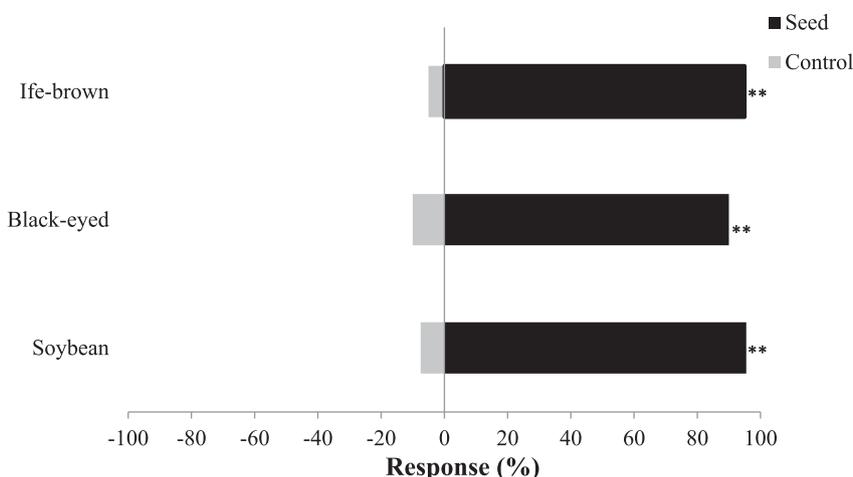


Fig. 1. Response of female *C. maculatus* to seeds of Ife-brown (IB), black-eye (BE) cowpea, and soybean (SB) compared to control in a separate bioassays (n = 40). ***: P < 0.0001 (two-sided binomial test; $\alpha = 0.05$).

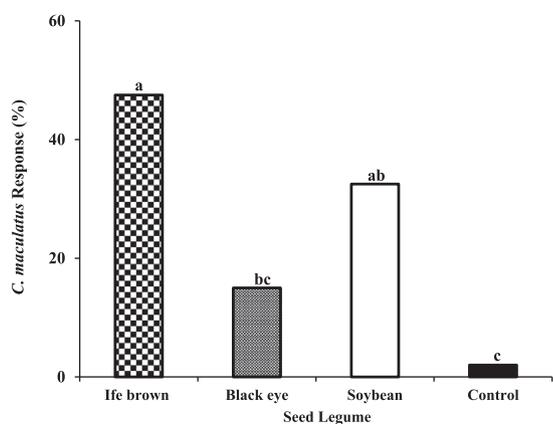


Fig. 2. Preferential attraction of *C. maculatus* to seeds of legume cultivars (Ife-brown cowpea, black-eyed cowpea and soybean) in four-choice olfactometer bioassays (n = 40). Means (with different letters are significantly different. (P < 0.05 (two-sided binomial test: $\alpha = 0.05$)).

and preference in this important pest. Being a field-to-store pest suggests that dispersing individuals are guided by specific cues to their preferred hosts (Foster and Harris, 1997; Scheirs et al., 2003; Phillips et al., 1993,1996; Sharma and Fadamiro, 2003; Peter et al., 1989; Collins et al., 2007; Cox, 2004). Adult *C. maculatus* do not feed (Rees, 2004). They only mate, and the mated females oviposit on suitable hosts within the 12–14 day lifespan after emergence and die naturally (Rees, 2004; Ajayi and Adedire, 2003). We hypothesized that oviposition preference of female *C. maculatus* among seed legumes is mediated by seed-derived volatile semiochemicals. This study was conducted to test this hypothesis and identify the semiochemical cues that mediate host preference of *C. maculatus* among seeds of three common grain-legume cultivars. It is hoped that the identified semiochemical attractant will offer potential for exploitation as lures for monitoring and control of *C. maculatus*.

2. Materials and methods

2.1. Insect and seed legumes

A culture of *C. maculatus* was reared in an insect rearing chamber maintained at 28 ± 1 °C, $65 \pm 5\%$ RH, and 12:12 h

photoperiod (Ajayi and Adedire, 2003). Mated females (1–2 day old) that emerged from a fresh culture were used for the bioassays. The weevils were sexed using the keys described by (Rees, 2004). Two cultivars of cowpeas (*V. unguiculata*): Ife-brown (an improved cowpea cultivar in Nigeria) and black-eyed (a cosmopolitan cultivar) were obtained from grocery stores (in Akure, Nigeria and Auburn, AL, USA, respectively), while soybean, *Glycine max* (Pioneer 95Y70 cultivar) was obtained from the EV Smith Research Center, Shorter, Alabama, USA. The seeds used in the bioassays were clean and uninfested by insects.

2.2. Olfactory response of *C. maculatus* to seeds of legume cultivars: Y-tube bioassays

First, a Y-tube olfactometer (Analytical Research Systems, Gainesville, FL) was used to test the behavioral response of *C. maculatus* to seeds of each legume cultivar (Ife-brown cowpea, black-eyed cowpea and soybean). The apparatus and procedures were modified after Chen et al. (2009) and Balusu and Fadamiro (2011). Briefly, the Y-tube olfactometer has two arms, one was connected to a jar that housed a legume seeds (1000 g) of a test cultivar and the second arm was connected to a jar that has no seed (control). Air was pushed in at a constant rate of 200 ml/min and removed via a vacuum pump at the rate of 450 ml/min. Light was switched off to create a dark environment similar to the situation in storage structures. An individual weevil was released and allowed 15 min to make a choice among the two arms. Weevils that did not walk into any of the arms within 15 min were scored as “non-responders,” and were not included in the analysis. After testing five weevils, each odor source was replaced with fresh sample. The olfactometer was cleaned with hexane and acetone after testing 20 insects and the arms were rotated (90°) to minimize positional effect. Each weevil was tested once and discarded. A total of 40 responders were used in analysis.

2.3. Olfactory response of *C. maculatus* to seeds of legume cultivars: four-choice bioassays

To determine host preference, attraction of *C. maculatus* to seeds of the three legume cultivars was compared using a four-choice olfactometer (Analytical Research Systems, Gainesville, FL). This apparatus consists of a central chamber (30 cm long by 30 cm wide by 6 cm high) with orifices or “arms” (17 cm long by 7 cm in

Table 1

Quantitative and qualitative analysis of headspace volatile organic compounds (VOCs) emitted by seeds of three legume cultivars. Table shows VOCs $\mu\text{g g}^{-1}$ seeds Means (\pm SEM) within the same row having different letters are significantly different ($P < 0.05$). Kovats retention index (KI) is indicated for each compound. nd = not detected. Data were collected from three biological replicates per treatment.

| VOCs | KI | Ife brown | Black eye pea | Soybean |
|--------------------------------|------|-------------|---------------|-------------|
| Hexyl formate | 981 | nd | nd | 0.29(0.01) |
| 1-Octen-3-ol | 972 | nd | nd | 0.23(0.02) |
| Unknown | | 0.31(0.02)a | 0.31(0.01)a | 0.32(0.03)a |
| 2-Ethyl-1-hexanol | 990 | 0.54(0.01)a | 0.23(0.01)b | 0.21(0.01)b |
| 1-hexanal | 806 | 0.31(0.02)a | 0.30(0.02)a | nd |
| 1-Decanol | 1263 | 0.34(0.03)a | 0.29(0.02)a | nd |
| 3-Ethyl-o-xylene | 1119 | 0.38(0.01) | nd | nd |
| 3,5-Octadien-2-one | 1072 | 0.34(0.01)a | 0.30(0.01)a | nd |
| n-Nonanal | 1104 | 0.74(0.03)a | 0.37(0.03)b | 0.19(0.10)c |
| Prehnitene | 1133 | 0.90(0.03) | nd | nd |
| 1-Vinyl-2,4-dimethylbenzene | 1105 | 0.37(0.01) | nd | nd |
| 1-butanol | 662 | 0.71(0.02)a | 0.31(0.15)b | nd |
| Unknown | | 0.45(0.06)a | 0.37(0.03)a | nd |
| 1-Menthol | 1164 | 0.57(0.24)a | 0.31(0.19)b | 0.31(0.02)b |
| Unknown | | 0.66(0.02)a | 0.54(0.20)b | nd |
| Decanal | 1204 | 0.25(0.12)a | 0.21(0.11)a | nd |
| 1-pentanone | 2636 | 0.22(0.11)a | 0.39(0.10)a | nd |
| Unknown | | 0.37(0.01)a | 0.34(0.20)a | nd |
| 1-Methylnaphthalene | 1345 | 0.74(0.03)a | 0.37(0.21)b | nd |
| Unknown | | 0.33(0.02)a | 0.35(0.03)a | nd |
| Unknown | | 0.36(0.01)a | 0.29(0.01)a | 0.30(0.01)a |
| Decyl ether | 2085 | 0.32(0.01)a | 0.22(0.11)b | nd |
| α -Cedrol | 1543 | 0.44(0.04)a | 0.21(0.10)b | nd |
| Tetradecane | 1413 | 0.40(0.06)a | 0.34(0.01)a | 0.35(0.05)a |
| Unknown | | 0.40(0.01)a | 0.32(0.02)a | nd |
| Naphthalene | 1231 | 0.56(0.15)a | 0.31(0.01)b | nd |
| Nerolidyl acetate | 1421 | 0.28(0.14)b | 0.49(0.18)a | 0.29(0.01)b |
| 9-Methyl-cis-decalin-1,8-dione | 1517 | 0.48(0.01)a | 0.19(0.10)b | nd |
| Nonadecane | 1910 | 0.53(0.01)a | 0.33(0.01)b | nd |
| Hexadecane | 1600 | 0.34(0.19)a | 0.29(0.01)a | nd |
| Unknown | | 0.29(0.16) | nd | nd |

diameter) at the four sides and a central orifice where mixing of the airflow from the arms occurred. The orifices were connected through Teflon-glass tube connectors to four air-tight glass jars (5 L) (Analytical Research Systems, Inc., Gainesville, FL, USA) with lids, which housed the test seeds. Each glass jar was provided with an inlet at the bottom and an outlet at the top of the lid and connected through Teflon tubing and ChemTred (8 mm i.d.) connectors to a flow meter on an air delivery system (ARS Inc., Gainesville, FL), which was again connected to an air source fitted with charcoal filter. The inlet air was further purified (using a second set of charcoal filters placed between the flow meters and the glass chambers), and pushed at a constant rate of 200 ml/min through the headspace of the test seeds in the glass jars into the orifices, and removed by suction via a vacuum pump through the central orifice of the olfactometer at the rate of 900 ml/min. Light was switched off to create a dark environment similar to the situation in storage structures. Seeds (1000 g) of each cultivar were placed in each glass jar. The fourth glass jar (without seed) served as the control. An individual beetle was released and allowed 15 min to make a choice among the four arms. Weevils that did not walk into any of the arms within 15 min were scored as “non-responders,” and were not included in the analysis. After testing five weevils, odor sources were changed. The olfactometer was cleaned with hexane and acetone after testing 20 insects and the arms were rotated (90°) to minimize positional effect. An individual weevil was tested once and discarded. A total of 40 responders were used in analysis.

2.4. Collection of headspace VOCs from seeds of legume cultivars

Headspace volatile organic compounds (VOCs) were collected by placing seeds (1000 g) of each test cultivar into a 5 L air-tight glass jar (Analytical Research Systems, Inc., Gainesville, FL, USA). A

volatile collection trap (8 cm long, 6 mm diameter) containing 80/100 mesh Super Q adsorbent was connected to the jar to trap the VOCs. A purified stream of air at a constant rate of 1 L/min was passed through the jar at room temperature (25 ± 1 °C) for 24 h. VOCs were eluted with 200 μL of methylene chloride and the resulting extract was stored in a freezer at -20 °C until used. The experiment was replicated six times.

2.5. GC analysis of VOCs

One μL of each headspace VOC extract was injected into a Shimadzu GC-17A equipped with a flame ionization detector (FID). The dimension of the capillary column used was as follows: HP – 5MS, 0.25 mm I.D., 0.25 μm film thickness (Agilent Technologies Inc. Santa Clara, CA, U.S.A). Helium was used as the carrier gas at a flow rate of 1 ml/min. The GC oven was programmed as follows: injection was at 40 °C hold for 2 min, which increased at the rate of 5 °C/min to 200 °C. The temperature of both injector and detector was set at 200 °C.

2.6. Coupled gas chromatography-electroantennogram detection (GC-EAD) analysis

Each headspace VOC extract was further subjected to GC-EAD analyses with the antenna of mated female *C. maculatus* to detect biologically active peaks (components) (Chen et al., 2009; Balusu and Fadamiro, 2011; Steidle et al., 2001; Fadamiro et al., 2010). The system consists of a Shimadzu GC-17A equipped with a FID coupled to an EAG detector. The dimension of the GC capillary column was the same as described above. The GC oven program was modified as follows: injection was at 40 °C hold for 2 min, which increased at the rate of 10 °C/min to 110 °C, 5 °C/min to

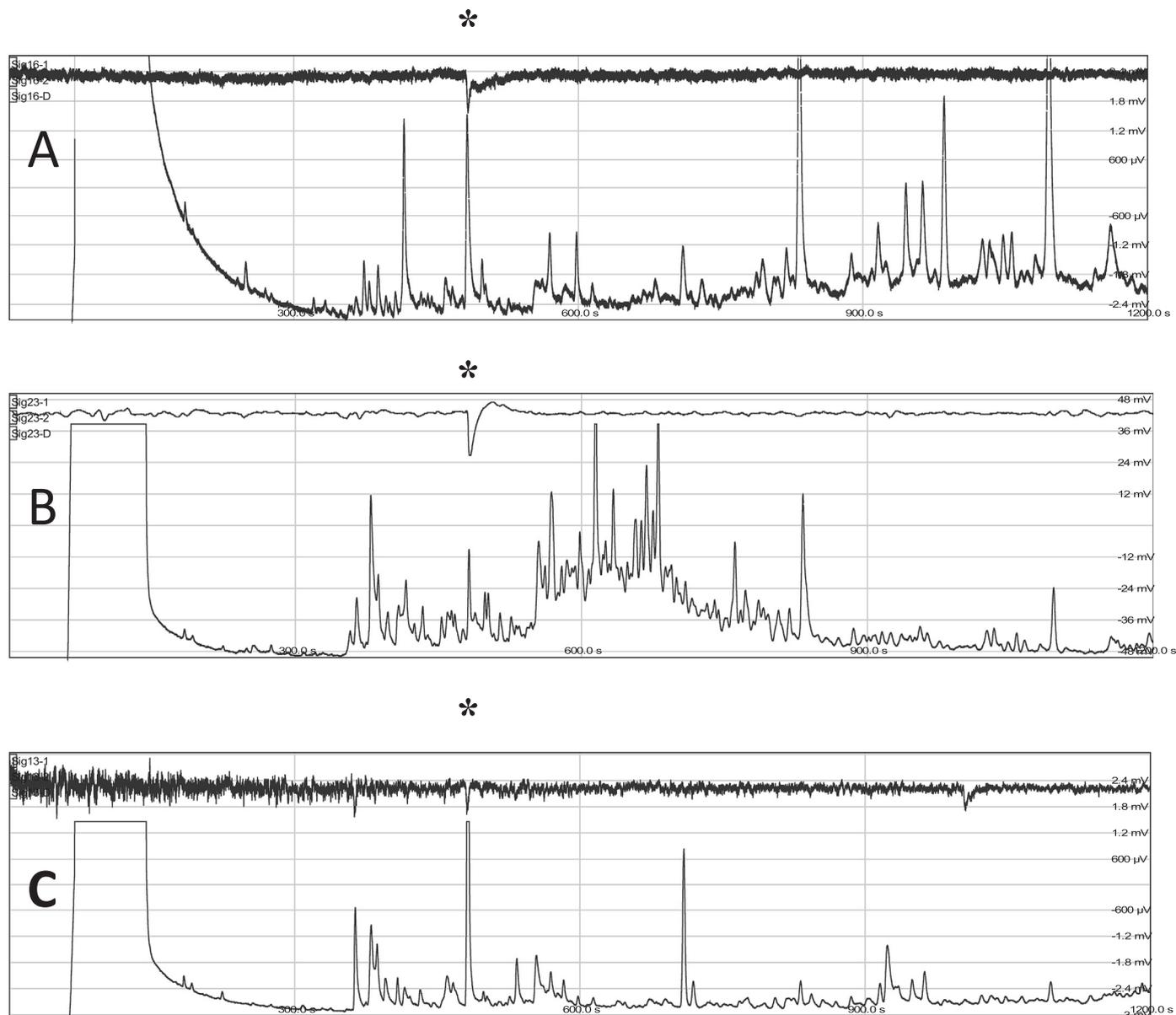


Fig. 3. GC-EAD responses of *C. maculatus* mated female to headspace VOCs emitted by seeds of legume cultivars: lfe-brown cowpea (A), black-eyed cowpea (B), and soybean (C). * indicates the GC-EAD active peak.

160 °C, and 20 °C/min to 200 °C with 2 min final hold time. The column effluent was split at a ratio of 1:2 (v/v), with one part going to the FID and the other through a heated (220 °C) transfer line (Syntech®, The Netherlands) into a humidified air stream (800 ml/min) directed at the antennal preparation (EAG detector). The antennal preparation and EAG techniques were done as previously described by [Chen and Fadamiro \(2007\)](#). Glass capillaries (1.1 mm I.D.) filled with Ringer solution were used as electrodes. The reference electrode was connected to the neck of the isolated head of female weevil, while the recording electrode was connected to the antennal tip (with part of the last antennal segment cut off). Chlorinated silver–silver chloride junctions were used to maintain electrical contact between the electrodes and input of the pre-amplifier (1 ×). The analog signal was detected through a probe (INR-II, Syntech®), captured and processed with a data acquisition controller (IDAC-4, Syntech®), and later analyzed using GCEAD Pro

software (Syntech®, Hilversum, The Netherlands) on a personal computer. A 3 μ L aliquot of each sample was injected for a GC-EAD run. At least three successful GC-EAD recordings were obtained for each treatment (i.e. legume cultivar), and traces were overlaid on the computer monitor to determine which GC peaks consistently yielded EAD responses.

2.7. GC–MS analysis

The GC-EAD active peak was identified by gas chromatography–mass spectrometry (GC–MS) using an Agilent 7890A GC coupled to a 5975C Mass Selective Detector, with an HP-5 MS capillary column (30 m × 0.25 mm I.D., 0.25 μ m film thickness). Compounds were identified by comparison of their mass spectra and retention indices (Kováts index) with those of reference substances and by comparison with the NIST mass spectral search software v 2.0 using

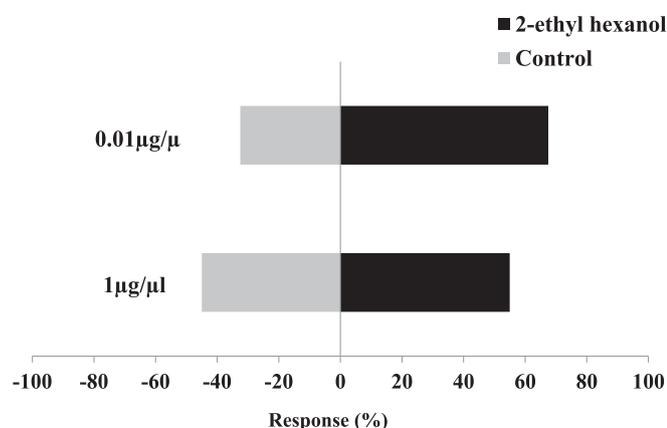


Fig. 4. Response of *C. maculatus* to synthetic 2-ethyl-1-hexanol at two concentrations (0.01 µg/µl and 1 µg/µl) in Y-tube olfactometer bioassays. Control = methylene chloride. (n = 40 per concentration. $P < 0.05$ (two-sided binomial test; $\alpha = 0.05$).

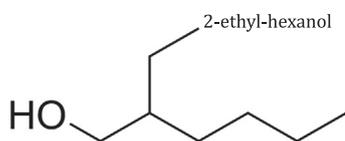


Fig. 5. Structure of electrophysiological active compound in volatile organic compounds of the test legume seeds.

the NIST 05 library (National Institute of Standards and Technology, Gaithersburg, MD, USA). External calibration curves were made with standard solutions of 1-menthol and pentadecane for quantitative measurements, as previously described in (Zebelo et al., 2014).

2.8. Response of *C. maculatus* to synthetic GC-EAD active compound

GC-EAD and GC-MS analyses identified 2-ethyl hexanol as the bioactive compound in the headspace VOCs of seeds of all three legume cultivars. To determine behavioral activity of this compound, pure synthetic 2-ethyl hexanol was purchased from Sigma[®] Chemical Co. (St Louis, Missouri). The compound was formulated in methylene chloride at 0.0001 µg µL⁻¹ and 0.01 µg µL⁻¹ concentrations, and delivered as 100 µl samples on Whatman No. 1 filter paper strips (30 × 20 mm). Attraction of mated female *C. maculatus* to two doses (low: 0.01 µg and high: 1 µg) of 2-ethyl hexanol was tested in Y-tube olfactometer as described above. In each experiment, the compound was introduced into one arm of the olfactometer, while methylene chloride was introduced into the other arm as solvent control. The two doses were selected based on preliminary experiments which showed that the beetles were not responsive to this compound at doses below 0.01 µg. All bioassays were conducted at 28 ± 2° C, 70 ± 5% relative humidity.

2.9. Statistical analysis

Data obtained in the Y-tube tests (attraction to seeds or synthetic compound) were analyzed using a two-sided binomial test. Host preference of *C. maculatus* among legume seeds in four-choice olfactometer was modeled as a binary response count and treatments were compared using Logistic Regression Analysis (Morawo and Fadamiro, 2014). The model adequacy for each set of

experiment was confirmed with a Likelihood Ratio (Wajnberg and Haccou, 2008). Slopes were separated using Proc Logistic Contrast in SAS. All analyses were performed using SAS 9.2 at 0.05 level of significance.

3. Results

3.1. Olfactory response of *C. maculatus* to seeds of legume cultivars: Y-tube

Female *C. maculatus* showed significant ($P < 0.0001$) attraction to the seeds of all three legume cultivars in Y-tube tests compared with the blank control. Out of the 40 female weevils tested for each legume cultivar, 38, 36 and 37 chose the seeds of lfe-brown cowpea, black-eyed cowpea and soybean, respectively (Fig. 1).

3.2. Olfactory response of *C. maculatus* to seeds of legume cultivars: four-choice bioassays

In four-choice olfactometer tests, female *C. maculatus* were preferentially attracted to the seeds of lfe-brown cowpea compared with the other two cultivars ($\chi^2 = 24.45$, $df = 3$, $P < 0.0001$) (Fig. 2). About 50% of the female weevils chose lfe-brown cowpea compared with soybean (30%) and black-eyed cowpea (15%). While female weevils showed preference for lfe-brown over black-eyed cowpea ($\chi^2 = 9.02$, $df = 1$, $P < 0.0027$), weevils did not discriminate between lfe-brown and soybean ($\chi^2 = 1.86$, $df = 3$, $P < 0.1729$) in four choice olfactometer.

3.3. GC, GC-EAD and GC-MS analyses

GC analysis revealed both qualitative and quantitative differences in the seed VOC profiles of the test legume cultivars with a total of 32 detectable peaks (compounds) (Table 1). At least seven of these peaks were detected consistently in the headspace of all three cultivars. More peaks were detected in lfe-brown (30 compounds) compared with black-eyed cowpea (26 compounds) and soybean (8 compounds). In particular, 2-ethyl hexanol, 1-Methylnaphthalene, à-Cedrol, Naphthalene and Nonadecane were detected in significantly higher amounts in lfe-brown cowpea than in black-eyed cowpea (Table 1). *n*-Nonanal was detected in higher amount in lfe-brown than in the other two cultivars. Nerolidyl acetate was the only compound detected in higher amount in black-eyed cowpea than in lfe-brown cowpea or soybean. Two compounds, hexyl formate and 1-Octen-3-ol were uniquely detected in soybean. Only one peak consistently elicited significant GC-EAD activity in female *C. maculatus* (Fig. 3). Based on comparison of mass spectra and retention indices by GC-MS the biologically active peak was identified as 2-ethyl hexanol (Fig. 5). Emission of 2-ethyl hexanol was significantly higher (0.54 µg g⁻¹ seeds) in lfe-brown cowpea than in black-eyed cowpea (0.23 µg g⁻¹ seeds), or soybean (0.21 µg g⁻¹ seeds) (Table 1).

3.4. Behavioral response of *C. maculatus* to 2-ethyl hexanol

Female *C. maculatus* showed significant attraction (68%, $P = 0.0385$) to 2-ethyl hexanol at the lower dose (0.01 µg) in Y-tube bioassays. However, no significant attraction (55%, $P = 0.6358$) was recorded at the higher dose (1 µg) (Fig. 4).

4. Discussion

Female *C. maculatus* were highly attracted to the seeds of the tested three legume cultivars (lfe-brown cowpea, black-eyed cowpea and soybean) in Y-tube bioassays. However, they showed

a strong preference for seeds of Ife-brown cowpea in four-choice olfactometer bioassays. The differences in attraction of female *C. maculatus* to the odors of the legume cultivars confirm that the bruchids have varied preferences for different legume varieties and cowpea cultivars (Rees, 2004). Ife-brown cowpea was the most preferred seed legume in this study. Surprisingly, the beetle (commonly called cowpea weevil) showed significantly greater attraction to soybean than black-eyed cowpea. The observed differences in VOC profiles may have mediated the differential attraction of *C. maculatus* to the seeds of the three cultivars (Smith, 1998; De Bruyne and Baker, 2008). These results are in agreement with previous studies showing that Ife-brown is among the most susceptible of cowpea cultivars to *C. maculatus* infestation. Ife-brown cowpea is currently a reference standard in cowpea-*C. maculatus* susceptibility tests at the International Institute of Tropical Agriculture Ibadan, Nigeria (Adedire and Ajayi, 2003; Obadofin, 2014; Mbata, 1993; Uddin and Adesiyun, 2012). It could be inferred from our results that oviposition preference in *C. maculatus* among seed legumes is mediated by seed VOCs.

Out of the total 32 detectable peaks in the headspace of seeds of the three legume cultivars only one peak elicited significant GC-EAD activity in female *C. maculatus*. The biologically active peak was identified by GC-MS as 2-ethyl hexanol. This compound was detected in greater amounts in Ife-brown cowpea, which was the most attractive cultivar in behavioral bioassays.

Our result which identified 2-ethyl hexanol as the lone biologically active compound is a bit surprising given that natural odors typically exist as blends or mixtures of compounds. Several studies have showed that VOC blends are more attractive to insects than individual components (Kessler and Baldwin, 2001; McComic et al., 2012; Hammack, 2001; Siderhurst and Jang, 2010; Beck et al., 2012; Li et al., 2012), and VOCs that are not attractive individually may contribute to olfactory contrast that enhances attraction to the most active compounds in a blend (D'Alessandro et al., 2009). However, there are a few examples of a single compound attractant for many insect species including z-3-hexenylviolanoside that attracts predatory insects to tomato pests (Mescher and De Moraes, 2014), dipropylsulfide attracts flies to onions (Dindonis and Miller, 1981), and isothiocyanate that attracts crucifer-feeding insects (Vincent and Stewart, 1984). 2-ethyl hexanol has been detected in peanut extracts and in the headspace of benzothiadiazole and jasmonic acid-treated Lima bean (*Phaseolus lunatus*) (Yi et al., 2009). It was also shown to elicit significant electrophysiological responses in *Cryptolestes ferrugineus* (D'Alessandro et al., 2009). Thus, it appears that this compound is fairly ubiquitous in nature but more commonly released by leguminous plants. It is plausible that a pest of legume crops such as *C. maculatus* will use this compound (2-ethyl hexanol) as a host location cue. Our results showed that host preference and discrimination among legume cultivars is achieved primarily by the observed quantitative differences in the emission of this compound by the various cultivars.

n-Nonanal was detected in higher amounts in the headspace of Ife-brown seeds than in black-eyed cowpea and soybean. Generally, *n*-nonanal has been reported as attractive to beetles (Siderhurst and Jang, 2010). Future studies will test mixtures of 2-ethyl hexanol and *n*-Nonanal at varying ratios and concentrations as an attractant blend for *C. maculatus*.

5. Conclusion

This study demonstrated that attraction of *C. maculatus* to legume seeds is mediated by semiochemicals. The results showed differential attraction of *C. maculatus* to seeds of legume cultivars. Of the three legume cultivars tested in this study, Ife-brown cowpea (a popular cowpea cultivar in Nigeria) was the most attractive to

C. maculatus. GC-MS and GC-EAD analyses of headspace VOCs emitted by legume seeds identified 2-ethyl hexanol as an attractant for *C. maculatus*. Future studies are necessary to optimize this attractant as a lure for managing *C. maculatus* in stored legume seeds.

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