

## Host seed type and volatile compound abundance level mould host location and preference behaviours in *Callosobruchus maculatus* (Fabricius, 1775) (Coleoptera: Chrysomelidae)

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**Abstract:** Dry seeds of cowpea, an important food, and cash crop to farmers, are heavily infested by *Callosobruchus maculatus* (Fabricius, 1775) during storage, causing huge economic loss. As a result, farmers spray pesticides on their harvest to control the pest attack with little consideration for the consequences of their actions. Due to health and environmental concerns associated with pesticide applications, farmers, marketers, and end-users are seeking alternative safer routes to handling this infestation problem. Thus, this study investigated the response of mated female *C. maculatus* to odour cues from different bean types using two-arm and four-arm olfactometers. The volatile organic compounds from the preferred beans (Borno brown and black-eyed beans-cultivars of *Vigna unguiculata* Walper, 1843 and adzuki bean – *Vigna angularis* (Willdenow) Ohwi & Ohashi, 1969), were analysed using gas chromatography-mass spectrometry (GCMS) techniques and headspace volatile organic compounds were tested in 2-arm olfactometer with the view to identifying possible attractants or deterrents that could be used in effective control of storage pest. The results indicated that (a) the female *C. maculatus* responded discriminatively to odour stimuli from the bean types tested, (b) eighteen volatile compounds were present in the bean types tested and (c) the volatile compounds identified varied in abundance profile. These suggest that host location and selection behaviours by female *C. maculatus* are moulded by the types and concentrations of the volatile compounds present in the beans.

**Keywords:** Bean, *Callosobruchus maculatus*, cowpea plants, odour cues, volatile compounds

### Introduction

Studies on how insects relate with their hostplants have revealed the prospects of managing pests' attacks using semiochemical-based approach (Cai *et al.* 2015). Odour cues detected over a distance drive many insect-plant interactions and many of the chemicals involved are volatile organic compounds (VOCs) (Dudareva *et al.* 2004). These substances can be released from the flowers, developing pods or seeds of a hostplant, and are used by insect pests to identify, home-in on and utilise a preferred host type (Ignacimuthu *et al.* 2000, Uechi *et al.* 2007, Webster *et al.* 2008).

The use of plant VOCs in pest control has produced some remarkable outcomes (Agelopoulos *et al.* 1999). For example, the cosmopolitan granary pest *Acanthoscelides obtectus* (Say, 1831), the pea beetle *Bruchus pisorum* (Linnaeus, 1758) and the legume pod borer (*Maruca vitrata*, Fabricius, 1787) are attracted to volatile compounds from dry bean cultivars (Khelfane-Goucem *et al.* 2014), *Pisum sativum* (Linnaeus, 1753) (Ceballos *et al.* 2015) and *Vigna unguiculata* (Bendera *et al.* 2015; Zhou *et al.* 2015), respectively. This attraction has been used to control infestations on these mentioned crops. A range of volatile blends as well as a single compound and variations in chemical profiles have been suggested to influence host discrimination in many insects

(Smith 1998, De Bruyne & Baker 2008). A study by Bruce & Pickett (2011), showed that insects use a combination of 3-10 compounds as chemical cues during host location. In another study, Bruce *et al.* (2005) identified 3-octane and 1-octanol as volatile inducing compounds against insect pests of stored grains. According to Ajayi *et al.* (2015), *C. maculatus*, showed 90–95% attraction to three leguminous cultivars and identified 2-ethyl hexanol as a key volatile compound driving the responses. Arnold *et al.* (2012) also reported that higher concentrations of methyl silicate, a botanically derived compound, repelled a subgroup of inactive *C. chinensis* adults compared to active adults. Another study on *C. chinensis* revealed that tridecane, a volatile from cowpea seeds infested with fourth instar larvae repelled conspecific females (Babu *et al.* 2003).

In many insect species, female egg-laying behaviour determines host acceptance or preference and differs with populations (Carrière & Roitberg 1996) and other factors. Gravid female *C. maculatus* use a combination of chemical and physical cues associated with host seed-surface to discriminate among seeds of legume cultivars (Messina *et al.* 1987, Credland & Wright 1989) and has exhibited behavioural attraction to different legume cultivars. For example, females avoid beans that already have eggs and/or larvae (Messina *et al.* 1987), and such egg-laying behaviour is influenced, in part, by the presence of the “detering” pheromones of conspecifics (Messina *et al.* 1987, Shu *et al.* 1996). They also consider host surface texture (Cope & Fox 2003), host bean size (Beck & Blumer 2014) and egg-load on a bean (Messina *et al.* 1987) when choosing an oviposition substrate.

Furthermore, *C. chinensis* was suggested to be attracted to volatiles from un-infested and egg-carrying seeds of cowpea and repelled by seeds carrying developing larvae (Ignacimuthu *et al.* 2000). Geographical location, beetle sex and morph also affect host preference in *Callosobruchus spp.* Messina & Slade (1997) have reported that egg-laying female

*C. maculatus* from Africa preferred cowpea to mung bean as an oviposition substrate, whereas strains from Asia could not distinguish between host types. Understanding the connection between the preference behaviour of this stored-product beetle towards host plants and identifying the VOCs responsible for such response would be an important step toward designing novel management strategies that will focus on monitoring, predicting, and controlling infestation outbreaks.

To examine how female *C. maculatus* uses olfactory cues in host selection during oviposition, their behavioural responses when exposed to odour from both suitable and unsuitable host types were examined. This study was driven by the notion that behavioural attraction and preference for a bean type by female *C. maculatus* is mediated by host odour cues and aims at examining the preferences of the mated female *C. maculatus* for odour from different agriculturally important bean types, identifying and quantifying candidate headspace volatile compounds from preferred host types and analysing the volatile compounds to identifying compounds that are more abundant on the various bean types.

## Materials and Methods

### Study location

This study was conducted at the laboratories (the insectary, GC\_MS lab and AWEC building) of the Department of Animal and Plant Science, University of Sheffield, United Kingdom, S10 2TN.

### Procurement of insects and bioassay

A wild strain of *C. maculatus* was collected from infested Borno-brown cowpea obtained from a farmer’s field in Taraba State, Nigeria. The infested seeds (from Nigeria) were taken to the insectary (the study lab) where they were incubated and monitored until the adults

emerged. The emerged strain was cultured in breeding containers (17x11.5 cm) with 200 g of uninfested whole Borno-brown bean. The container lids were perforated to aid ventilation and the cultures kept in a laboratory at a relative humidity of  $30\pm5\%$  and  $28\pm2^\circ\text{C}$  temperature.

### *Bean seeds*

Seeds of five bean types were used in this study: Borno brown and black-eyed bean (cultivars of *Vigna unguiculata* L. Walper), adzuki bean (*Vigna angularis* Wild), mung bean (*Vigna radiata* Wilczek, 1954) and pinto bean (*Phaseolus vulgaris* Linnaeus, 1753). Except for "Borno brown" from Nigeria, all were sourced from a local Whole Food Store (in Sheffield, United Kingdom).

### *Responses of female C. maculatus to odour cues from different bean types in a four-arm olfactometer*

To examine the preference of the beetle to odour from a mixture of beans, seeds of three bean types were used: A familiar host (Borno-brown), an unfamiliar bean of the same genus (adzuki bean) and another unfamiliar host of a different genus (pinto bean). This bean choice is not unrealistic in field situation, especially in African mix-cropping system, where the beetle is often faced with a wide range of host types. A four-arm olfactometer with three layers (floor, observation, and cover) was used. The floor was fitted with a Whatman filter paper (110 mm) to provide traction while the observation layer had four edges drilled into the four arms of the olfactometer. A hole (4 mm diameter) was also drilled at the centre of the third layer (cover) for air suction. Four (60 ml) BD plastipak's were used as odour chambers. Each of the bean types was placed in one of the odour chambers, while the fourth chamber served as a control (clean air). A polytetrafluoroethylene (PTFE) connecting tubing (1.5 mm ID x 3.2 mm OD) was used to link each of the chambers to the four arms of

the olfactometer, and the connections sealed with PTFE tape. The four odour chambers were surrounded with brown paper to prevent the beetle from viewing the samples. A 60 W light bulb was positioned above the olfactometer to provide illumination. A mated (2 days old) female was introduced (with a Pooter) into the observation arena, and airflow was generated using a vacuum air pressure pulling air through the four arms of the olfactometer at a rate of 200 ml/min. After the beetle's introduction, it was given 3 min to acclimatise before being allowed to make a choice (for 15 min.). Beetles that made no decision within 5 min after introduction were discarded. After testing five beetles, the odour source was replaced. The olfactometer arm together with the filter paper was rotated after each test to reduce any positional effects. Each weevil was tested only once. Data on beetle response to odour were collected as the mean time spent in each odour chamber (arm). Each odour source was tested with 8–10 individuals.

### *Responses of female C. maculatus to odour cues from different bean types in a two-arm olfactometer*

To test the attraction of the beetle to odour from a particular bean, five different bean types were used: Borno brown, black-eyed bean, adzuki bean, mung bean and pinto bean. This approach was designed to measure the beetle's preference for a particular host which is a familiar situation in most storage conditions. A two-arm olfactometer was used for the study. It consists of three layers representing the base (floor), the observation layer and the cover clipped together to form an eight-sided shape with a two-arm exposure chamber. Each layer was made of a transparent Perspex base 6 mm thick. The first layer (floor) was lined with a Whatman filter paper base (110 mm) to provide traction for the beetle. Another layer, the observation arena had a hole (3 mm diameter) drilled from both edges into the two arms to accommodate

the odour chambers. Then, a third layer (cover), all the same size and shape, had a hole (4 mm diameter) drilled at the centre. Two 60 ml BD plastipak's (syringes) served as the odour chambers. A Teflon tube (1.5 mm ID x 3.2 mm OD) was used to connect each of the chambers to both arms of the olfactometer, and the connections were tightened with a PTFE tape. A bean type was placed into one of the odour chambers, while the second chamber was used as a control (clean air). Both chambers were covered with a brown paper to prevent the beetle from having a visual cue of the host. A 60 W light bulb was positioned 1 m above the olfactometer to provide uniform illumination. A mated female was introduced into the centre of the olfactometer (observation arena). Air was drawn through both arms using a vacuum air pressure, and regulated with a flow meter at a rate of 100 ml/min. After the introduction, each weevil was given 3 min to settle in the observation arena, and the movement towards both arms was observed for 15 min. Beetles that do not make any choice after 5 min of introduction were regarded as "non-responders" and discarded. The olfactometer arm together with the filter paper was rotated at intervals as described above after each test to reduce any positional effects. Each weevil was tested only once. Each odour source was tested with 8–10 individuals, and data on bruchid response were recorded.

#### *Collection of headspace VOC's from the preferred bean types*

The headspace collection of organic compounds released from seeds of the three bean types attractive to the beetle was carried out for a 24-hour period. A hundred (100) g of seeds of each bean type was placed in a glass vessel (190 mm high x 100 mm wide), open at the top for an inlet and outlet ports. A volatile collection trap (8 cm long, 5 mm diameter) containing Porapak Q absorbent (50 mg, 80/100 mesh) was connected to the glass

vessel to trap the VOCs. Charcoal filtered air was passed through the Porapak Q absorbent at a constant rate of 1 L/min. All the connections were made with PTFE tubing and tape. VOCs absorbed on Porapak Q were eluted with 1 ml of acetone. Extracted samples were stored in glass vials in a freezer at -80°C until used for analyses and bioassays.

#### *Beetle attraction to headspace volatiles from the preferred bean types*

To establish the attractiveness of the beetle to a bean type, the volatile samples collected were used in a two-arm olfactometer. Twenty microliters (20 µl) of volatile samples from the preferred bean types (Borno brown, adzuki bean and black-eyed bean) were applied on a piece of filter paper, and 1 min was allowed for solvent (hexane) evaporation. The treated filter paper was then put into one of the odour chambers, while the second chamber was used as a control which contained a piece of filter paper treated with 20 µl of hexane. A mated female was then introduced into the centre of the olfactometer (observation arena). Air was pulled through both arms using a vacuum air pressure and regulated with a flow meter at a rate of 100 ml/min. After introduction, each weevil was given 3 min to settle in the observation arena, and the movement towards both arms was observed for 15 min. Weevils that made no choice after 5 min of introduction were regarded as "non-responders" and discarded. Each beetle was tested only once, and the proportion of time spent in each arm was recorded.

### **General comments**

#### *Identification of volatile compounds*

Organic compounds in volatile bean samples were identified using Gas chromatography – mass spectrometry (GC-MS). A 2 µl of the air headspace sample was injected onto a capillary GC column (30 m x

0.25 mm ID, 0.25  $\mu$ m film thickness), which was directly coupled to a mass spectrometer (PerkinElmer, Clarus SQ 8T). Helium was used as the carrier gas with a flow rate of 1.02 mL min<sup>-1</sup>. Ionization was achieved by electron impact at 70 eV, 230°C. The injection port was maintained on a splitless mode. The GC initial oven temperature was maintained at 30°C/min, then ramped at 5°C/min to 240°C, and held for 20 min. Mass spectrum acquisition was scanned using a mass/charge ( $m/z$ ) range of 35 to 450. Candidate compounds were identified by comparing the chromatograph retention index and mass spectra with library database spectra using the National Institute of Standards and Technology (NIST) mass spectra search programme (version 2.2, NIST 14, Gaithersburg, Maryland, USA) (Babarinde *et al.* 2017). The retention index of each compound identified was calculated using a series of straight alkanes (C<sub>8</sub>–C<sub>20</sub>). The abundance of each identified compound was calculated by integrating the peak areas of the total ion chromatograph and averaged.

### Statistical analyses

The four-choice data on the beetles' responses to odour from different bean types was subjected to One-way ANOVA in a completely randomised design, whereas the two-arm result was analysed using Chi-square ( $\chi^2$ ) test, and 8-10 replicates were used. Stacked bars were used to present the proportion of time spent by the beetles in the two-choice olfactometer. The chemical analysis data on the abundance of volatile compounds from each bean type examined was subjected to perm-ANOVA analysis to identify variances amongst compounds and Tukey's HSD test was used to compare the mean differences. The similarities of the compounds based on their abundance were interpreted using cluster analysis (by Ward's Minimum Variance Cluster method); whereas principal component analysis (PCA) was used

to indicate the ordination and contribution of the compounds to the components.

## Results

### *Response of female C. maculatus to odour from bean types.*

The four-arm choice test showed that mated females did not discriminate between clean air and odour stimuli from adzuki bean and pinto bean. However, the insects responded to odour from Borno-brown significantly and spent more time ( $F=7.68$ ,  $df=3, 36$ ,  $p<0.001$ ) in the arm containing it (Fig. 1).

### *Attraction of female C. maculatus to host-bean odour*

The behavioural orientation of female beetles to odour from seeds of different beans is presented in Figure 2. When compared to clean air in each case, mated females spent significantly more time in the arm containing odour from adzuki bean ( $\chi^2=11.77$ ,  $df=1$ ,  $p<0.001$ ), black-eyed bean ( $\chi^2=10.98$ ,  $df=1$ ,  $p<0.001$ ) and Borno brown cowpea ( $\chi^2=5.28$ ,  $df=1$ ,  $p=0.022$ ). However, in the case of pinto beans ( $\chi^2=0.65$ ,  $df=1$ ,  $p=0.422$ ) and mung beans ( $\chi^2=2.51$ ,  $df=1$ ,  $p=0.113$ ), there was no statistical difference in time spent between bean odour and clean air arms.

### *Beetle attraction to headspace volatile samples*

Mated female beetles discriminated between bean odour and clean air and they spent significantly longer time in olfactometer arm containing volatile samples from Borno-brown ( $\chi^2=3.956$ ,  $df=1$ ,  $p=0.046$ ), black-eyed bean ( $\chi^2=5.581$ ,  $df=1$ ,  $p=0.018$ ) and Adzuki beans ( $\chi^2=4.219$ ,  $df=1$ ,  $p=0.039$ ). More than 50% of the observation time was spent in arms containing organic volatiles from beans (Fig. 3).

Table 1. Abundance (mean±SD) and retention index (RI) of the volatile organic compounds emitted by 2µl of air entrainment sample of Adzuki bean and Borno-brown bean Black-eyed bean.

Compounds	Adzuki bean	Borno-brown bean	Black-eyed bean	RI
Limonene	18.589±0.365 <sup>a</sup>	17.598±0.067 <sup>abcdef</sup>	17.840±0.374 <sup>abc</sup>	1030
Benzyl Alcohol	17.722±0.08 <sup>abcd</sup>	17.003±0.124 <sup>cdefghij</sup>	17.156±0.257 <sup>cdefgh</sup>	1036
Nonanal	17.670±0.287 <sup>abcde</sup>	16.920±0.060 <sup>cdefghij</sup>	18.387±1.472 <sup>ab</sup>	1104
Benzaldehyde	17.288±0.02 <sup>bcdefg</sup>	16.656±0.167 <sup>defghijkl</sup>	17.081±0.218 <sup>cdefghi</sup>	962
3-Carene	16.681±0.247 <sup>cdefghijkl</sup>	16.183±0.079 <sup>ghijklmno</sup>	16.534±0.193 <sup>efghijklm</sup>	1011
Propanoic acid, 2-Methyl,-3-hydroxyl-2,2,4-trimethylpentyl ester	16.526±0.294 <sup>efghijklm</sup>	16.085±0.247 <sup>hijklmno</sup>	16.710±0.005 <sup>cdefghijkl</sup>	1380
1-Hexanol, 2- Ethyl	16.075±0.423 <sup>hijklmno</sup>	15.536±0.230 <sup>lmnop</sup>	16.469±1.446 <sup>fghijklmn</sup>	1030
Pentanedoic acid, Dimethyl ethane	16.054±0.001 <sup>hijklmno</sup>	15.069±0.170 <sup>opqr</sup>	15.459±0.179 <sup>mnpq</sup>	1135
1- Nonanol	15.908±0.013 <sup>ijklmno</sup>	16.798±0.242 <sup>cdefghijk</sup>	17.043±0.026 <sup>cdefghij</sup>	1173
2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	15.861±0.503 <sup>ijklmno</sup>	15.419±0.256 <sup>mnpq</sup>	16.184±0.011 <sup>ghijklmno</sup>	1580
O-Xylene	15.635±0.02 <sup>klmnop</sup>	nd	15.689±0.002 <sup>klmnop</sup>	887
Napthalene	15.563±0.156 <sup>lmnop</sup>	16.088±0.048 <sup>hijklmno</sup>	15.292±0.027 <sup>nopq</sup>	1182
P-Cymene	14.601±0.426 <sup>pqrst</sup>	16.005±0.032 <sup>hijklmno</sup>	14.133±0.299 <sup>qrstu</sup>	1116
Benzene, 1,2,3,4-Tetramethyl	13.455±0.213 <sup>rtuvw</sup>	14.583±0.010 <sup>pqrst</sup>	12.275±0.129 <sup>w</sup>	1146
Hexanal	13.055±0.163 <sup>uvw</sup>	13.009±0.162 <sup>uvw</sup>	13.589±0.065 <sup>stuv</sup>	800
P-Xylene	12.895±0.274 <sup>vw</sup>	13.105±0.040 <sup>uvw</sup>	13.257±0.255 <sup>uvw</sup>	836
Naphthalene, 1,5-Dimethyl	nd	14.015±0.107 <sup>rstuv</sup>	15.030±0.029 <sup>pqrs</sup>	1440
2,4-Dimethyl-1-heptane	nd	16.950±0.033 <sup>cdefghij</sup>	14.655±0.042 <sup>pqrs</sup>	836

Means with the same letter(s) across rows are not significantly different ( $p>0.05$ ); nd – not detected; RI – Retention index.

### Identification and chemical analyses of volatile compounds

A total of eighteen (18) compounds were identified in black-eye beans while seventeen (17) and sixteen (16) compounds were identified in Borno-brown and adzuki beans, respectively (Table 1). Naphthalene, 1,5-dimethyl and 2,4-dimethyl -1-heptane were not detected in adzuki bean, whereas O-xylene was not detected in Borno-brown. The chemical analyses of the abundance profiles of the compounds indicated that they varied significantly ( $F=402.96$ ,  $df=17$ ,  $53$ ,  $p<0.01$ ) across the bean types tested, and a post-hoc test (Tukey's HSD) further revealed how they differed (Table 1). Limonene was the dominant compound, followed by benzyl alcohol and nonanal in adzuki bean; whereas, in Borno-brown bean, limonene was also dominant, followed by benzyl alcohol. Nonanal was the dominant compound in the black-eye bean,

followed by limonene and benzyl alcohol (Table 1). However, p-xylene was the least abundant compound in adzuki bean. Both p-xylene and hexanal were the least abundant in Borno-brown bean. Benzene-1,2,3,4-trimethyl was the least abundant in black-eye bean (Table 1). The abundance of the compounds also differed across the bean types: limonene, benzyl alcohol, benzaldehyde, 3-carene and pentanedoic acid, dimethyl ethane were more abundant in adzuki bean, followed by black-eyed bean and Borno-brown. Nonanal was higher in black-eyed bean, followed by Adzuki bean and Borne-brown. However, hexanal level does not vary between Adzuki bean and Borno-brown. Similarly, the abundance of p-xylene was the same in Borno-brown and black-eyed beans (Table 1). The cluster analysis grouped the compounds in three clusters. Limonene, representing cluster 1 has no similarity with any other compound (Fig. 4). Compounds in the same cluster share a similar

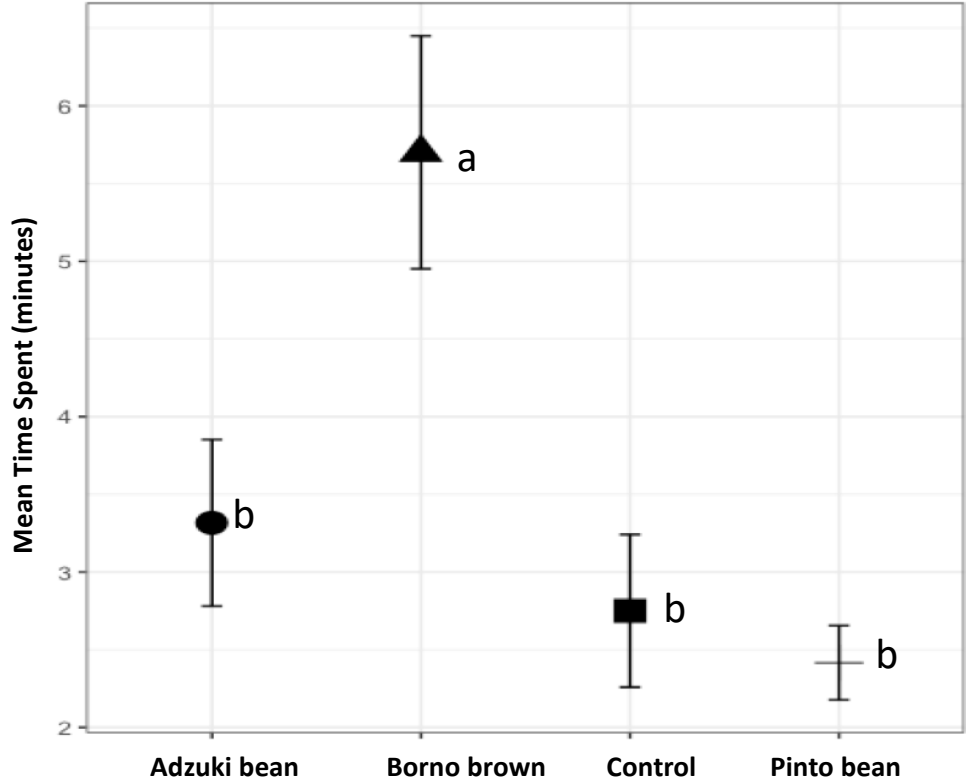


Fig. 1. Mean time spent by mated female *Callosobruchus maculatus* in response to odours from three bean types.

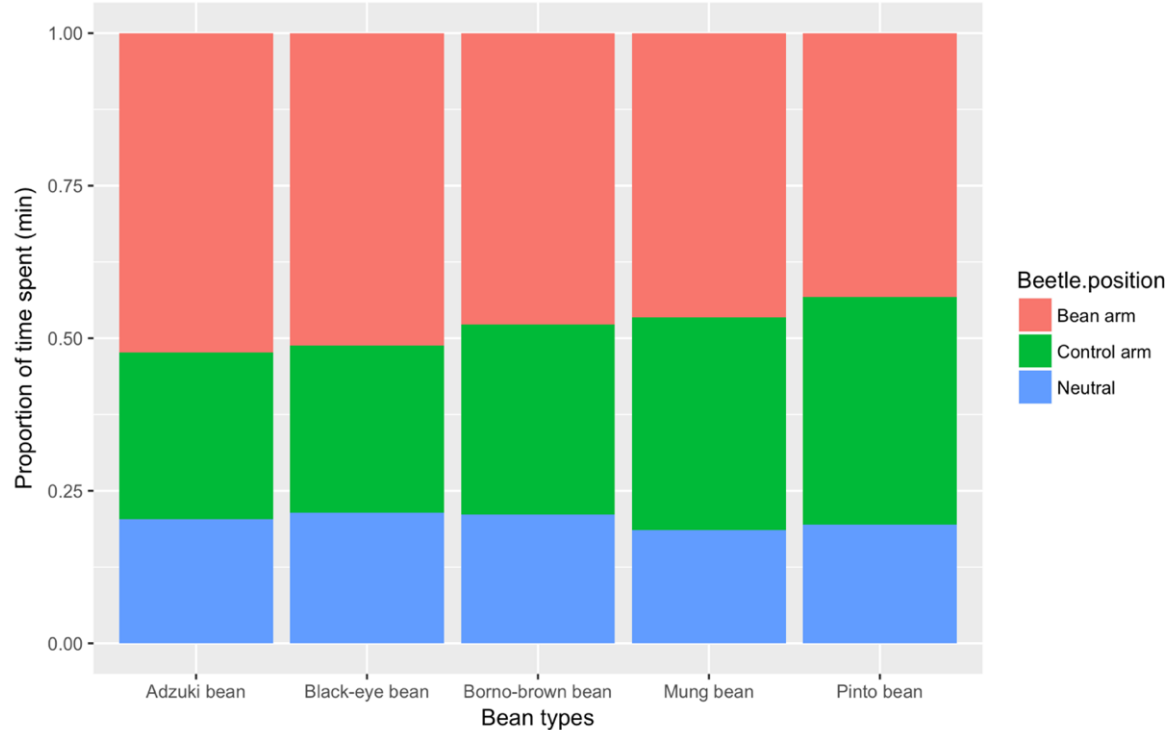


Fig. 2. Proportion of time spent by mated female *Callosobruchus maculatus* in response to volatile odours from seeds of legume cultivars compared to control in a two-arm olfactometer.

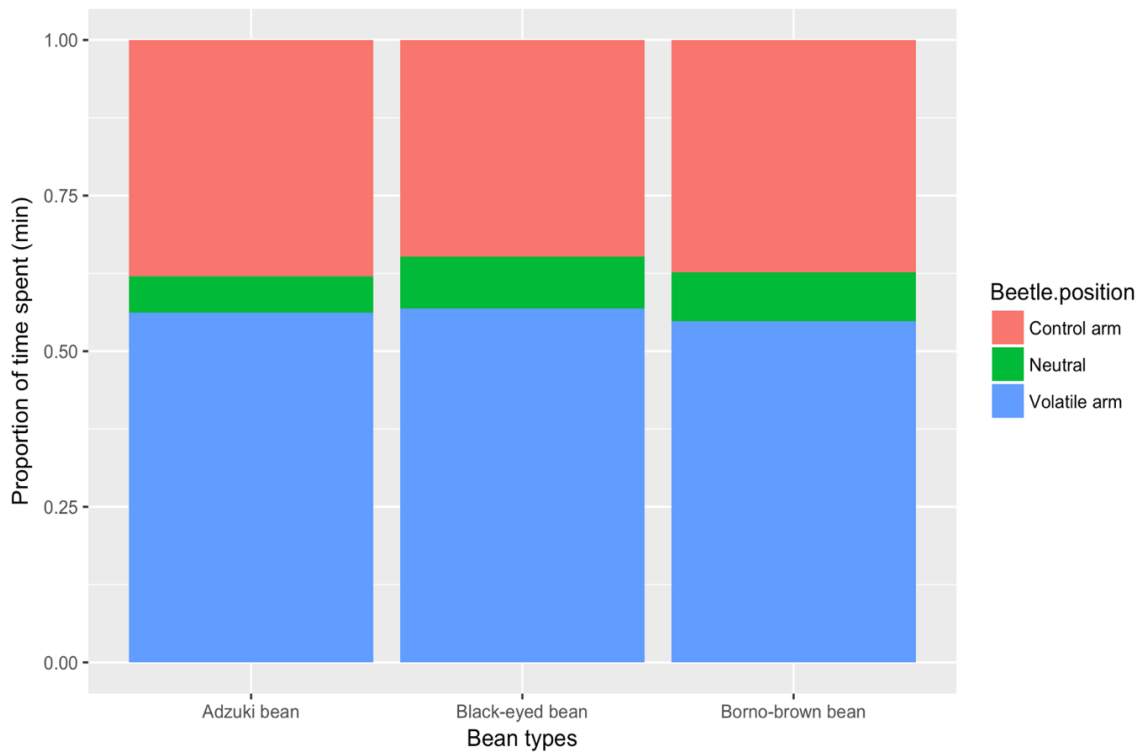


Fig. 3. Proportion of time spent by mated female *C. maculatus* in response to volatile samples from three bean types compared to control.

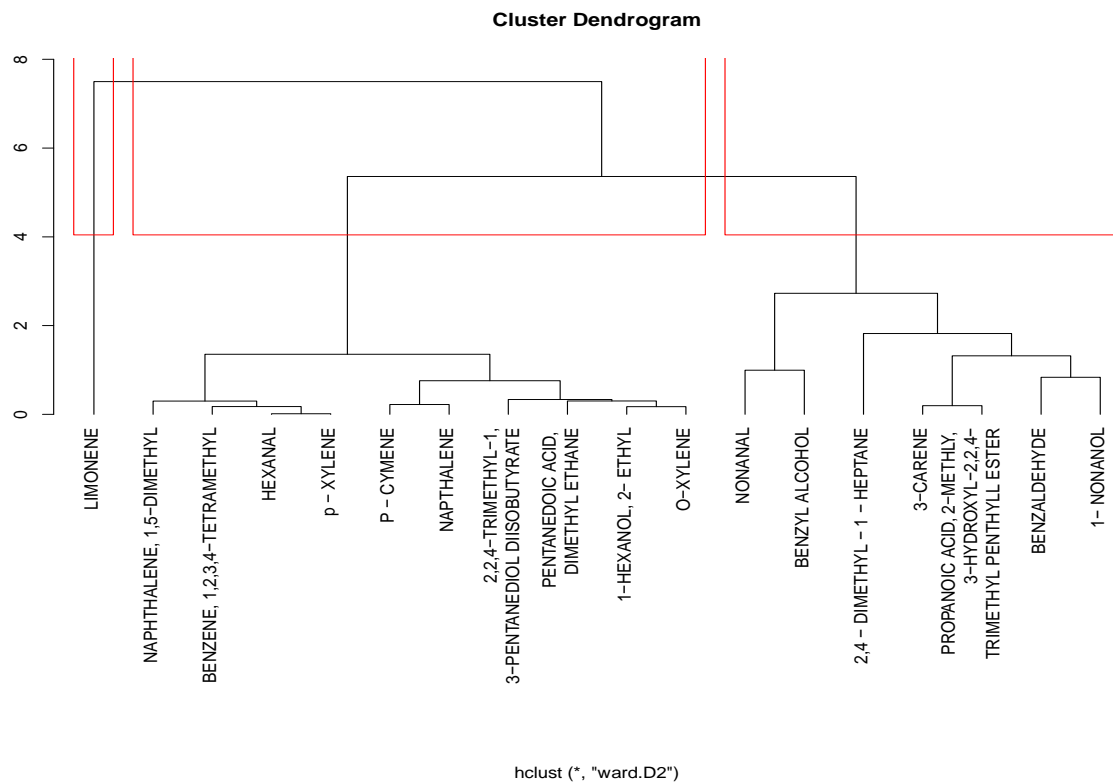


Fig. 4. Dendrogram showing relationship among 18 volatile compounds from three bean types based on their relative abundance. The rectangular boxes represent each cluster.



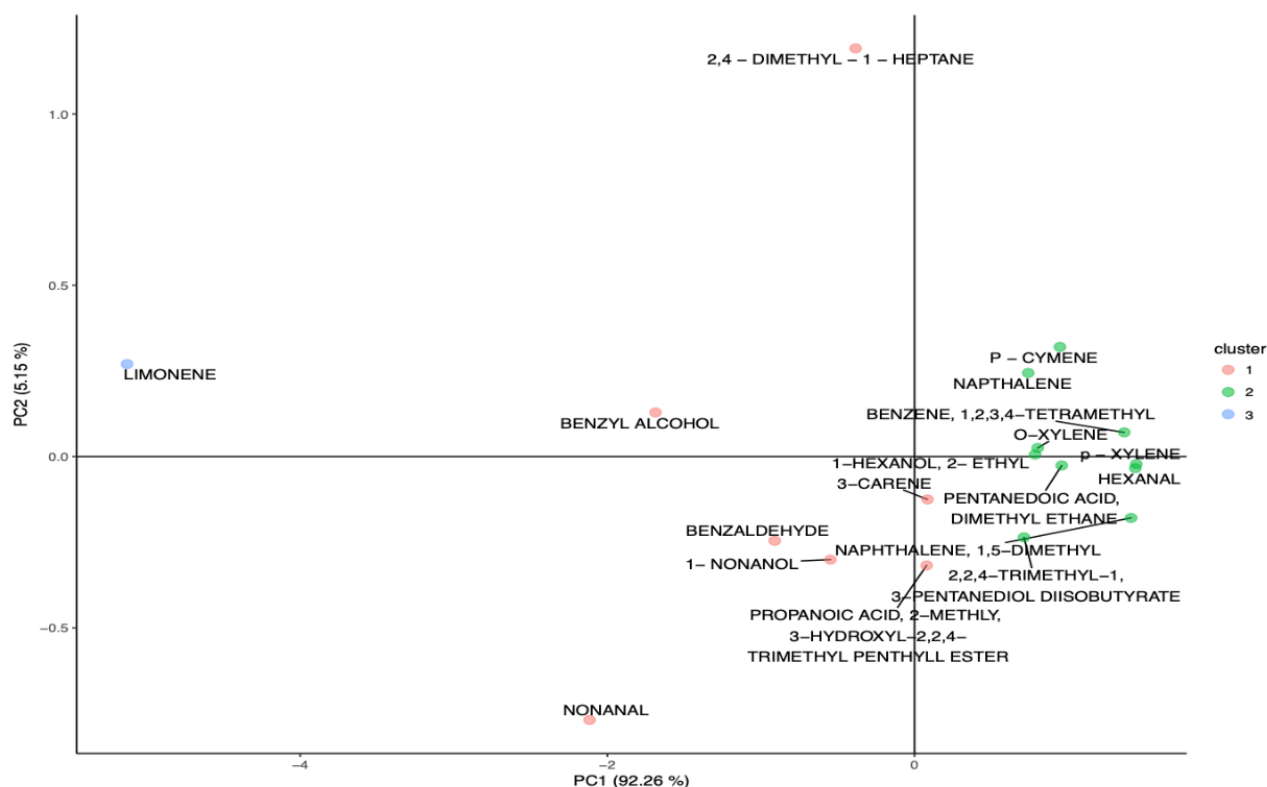


Fig. 5. Biplot showing the ordination, cluster and contribution of the volatile compounds in the principal components.

abundance profile. The PCA plot showed that components, 1 and 2 explained more than 97% of the variance in the abundance of VOCs examined (Fig. 5).

## Discussion

The results of the four-choice test indicated that mated female *C. maculatus* preferred Borno-brown beans to pinto beans and adzuki beans. The findings suggest that *C. maculatus* females prefer a primary (familiar) host when exposed to hosts from different leguminous cultivars. Ignacimuthu *et al.* (2000) have suggested that the strong response of *C. chinensis* to uninfested cowpea seeds indicates the presence of cowpea derived volatile attractant. Our finding agrees with the work of Arnold *et al.* (2012) which showed that *C. maculatus* was strongly attracted to cowpea odour. When the beetle was presented with two choices (clean air vs a bean odour), it showed a strong preference for adzuki beans,

black-eyed beans and Borno-brown beans, respectively. This is interesting because the beetle could not discriminate between clean air and Adzuki bean in the 4-arm olfactometer thus suggesting that the super-abundance of volatiles from Borno-brown beans might have created a confusion effect. Surprisingly, the beetle showed no preference for mung beans (an ancestral host) or pinto beans (an unsuitable host) over clean air. This suggests that the beetle may not detect (or respond to) cues from both bean types at a distance. The results of *C. maculatus* attraction to headspace volatile samples showed that they were attracted to the three bean types (Borno-brown bean, black-eyed beans and adzuki beans) tested. These findings confirm the beetle's preference for an alternative host when a familiar or most preferred host is not presented. It further indicates that the behavioural attraction of the beetle to the samples was induced by chemical stimuli as visual cues were excluded in the study. The

roles of visual, taste and olfactory cues in host location and discrimination by other insects have also been reported (Chapman 2012, Ndomo-Moualeu *et al.* 2016, Hudaib *et al.* 2017).

Eighteen volatile compounds associated with the headspace samples from the preferred bean types have been identified in this study, and most of the compounds have been reported to elicit attraction in red palm weevil (Gunawardena & Herath 1995), legume pod borer (Bendera *et al.* 2015, Zhou *et al.*, 2015) and cucujid beetles (Mushobozy *et al.* 1993). For example, Ajayi *et al.* (2015) identified thirty-one volatile compounds from seeds of 3 legume cultivars, whereas Adhikary *et al.* (2015) reported the presence of 23 compounds from the seeds of four varieties of *Lathyrus sativus*. The numbers and composition of compounds present in each bean type examined varied slightly (which could be due to the differences in the sequence of genes in the bean cultivars (Köllner *et al.* 2004), Limonene, benzyl alcohol and nonanal dominated the abundance profile of the volatile compounds, and the importance of these compounds in managing agricultural pests has been documented. For example, limonene and benzaldehyde were among the volatile compounds of cowpea that influenced the behaviour of *Maruca vitrata* (Zhou *et al.* 2015) and the granary pest, *A. obtectus* (Khelfane-Goucem *et al.* 2014). A synthetic blend of nonanal, linalool, 1-octanol, 3-octanol and 3-octanone elicited behavioural attraction of *C. maculatus* (Adhikary *et al.* 2015). Benzyl alcohol has been reported to induce the attraction of natural enemies during insect pest infestation (De Moraes *et al.* 1998, Tabata *et al.* 2011), thus acting as a defensive compound. Also, hexanal is associated with the VOCs of *Pisum sativum* L. (Ceballos *et al.* 2015).

In summary, this study has demonstrated that the behaviour of female *C. maculatus* is influenced by odour stimuli associated with Borno-brown beans, black-eyed beans and adzuki beans. Limonene, benzyl alcohol and

nonanal were candidate compounds that could induce the beetles' behavioural attraction to the bean types and volatile compound composition and abundance profiles varied within compounds and among bean types. A follow-up study would focus on using electroantennography technique to examine the probable role of each compound already identified on the beetle's behaviour.

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