International Journal of Tropical Insect Science page 1 of 11
doi:10.1017/S1742758418000152

Preference of flight morph of *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) for three plant legumes

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(Accepted 11 April 2018)

Abstract. We studied host discrimination in flight morph of *Callosobruchus maculatus* (F.) among the legume plants Ife-brown (IB) and black-eyed (BE) cowpea, *Vigna unguiculata* (L. Walp), soybean (SB), and *Glycine max* (L.) and analysed legume volatiles towards developing an attractant for *C. maculatus*. Olfactometry studies were conducted to investigate attraction of mated 3–4-day-old female *C. maculatus* to legume plants with the green pod (PGP), green pods (GP), plant with the ripened pod (PRP), ripened pod (RP), and plant without pod (PWP). We also assessed the response of beetles to phenological stages of the most attractive legume. In Y-tube bioassays, *C. maculatus* showed greater attraction to individual legumes at three phenological stages: PGP (73–93%), PRP (80–100%), and RP (63–93%) compared with PWP (6–36%). In four-choice bioassays, *C. maculatus* preferred IB at all stages to other legumes. SB had an insignificant attraction on the beetle. The RP of IB was the most attractive part of the plant. Gas chromatography (GC) and GC–mass spectrometry (GC–MS) analyses of volatiles emitted by RP of the legumes revealed a variation in the compositions of odour blends. Benzaldehyde was found in the volatiles of IB and BE with a higher emission in IB. Octanone was detected only in IB. The two compounds were undetected in SB volatiles. These results reveal that in addition to infesting cowpea plants at RP stage as reported in previous studies, *C. maculatus* could also infest cowpea plants at the GP stage.

Key words: *Callosobruchus maculatus*, flight morph, legume cultivars, green pod, ripened pod, plant without pod, GC–MS, benzaldehyde, octanone

Introduction

The use of synthetic chemical pesticides is being discouraged due to problems such as the development of resistance and pest resurgence, toxicity to humans and other beneficial non-target organisms, and environmental pollution (Ngowi *et al.*, 2007). The limitations on the use of some pesticides and fumigants in stored products and the increasing public demand for pest-free food products necessitate the development of biorational strategies in pest management (Koul *et al.*, 2008).

Semiochemicals can mediate behavioural interactions between organisms in the environment (Price *et al.*, 2011; Farré-Armengol *et al.*, 2013). These chemicals mediate intraspecific (pheromones) and interspecific (allelochemical) interactions between organisms. Insects use semiochemicals to find mates, food, and oviposition sites (Burkholder, 1990; Foster and Harris, 1997; Phillips, 1997).

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Plants emit myriads of allelochemicals that insects explore as cues to locate their hosts for feeding and oviposition (Nishida, 2014). The selection of an oviposition site by an adult female insect is critical to the survival of her offspring, because larvae that emerge from the eggs are destined to feed on the selected host. Therefore, female insects are expected to lay their eggs with high precision (Thompson, 1998; Awmack and Leather, 2002; Scheirs *et al*., 2003). The knowledge of the role of semiochemical cues in insect–host-location behaviour has been explored in developing semiochemical-based management strategies for some insect pests (Phillips *et al*., 1993, 1996; Sharma and Fadamiro, 2013).

Cowpea (*Vigna unguiculata* L. Walp.) is an annual legume that originated in Africa and is widely grown in Africa and Southeast Asia (Abate *et al*., 2012). Cowpea is the primary protein-rich staple food and complements cereal grains and starchy tuber-crop-based diets (Duke, 1990). Insect pests have been a major drawback in the production of cowpeas, especially during storage. The cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae), is a pest of many pulses but a significant pest of *Vigna* spp. (Lale, 2002; Rees, 2004; Swella and Mushobozy, 2009), and the most important economic pest of cowpea, *V. unguiculata* (Adedire and Ajayi, 2003). *Callosobruchus maculatus* exists in dimorphic forms: the sedentary (flightless) and the active (flight) morphs (Utida, 1972; Sano-Fujii, 1984; George and Verma, 1999). The sedentary morph is highly fecund and attacks seeds during storage. The flight morph exhibits less fecundity and delayed reproduction and disperses from stores to lay eggs on the pods of host plants in the field (Messina and Renwick, 1985). The dimorphic nature of the flight form enhances dynamic cycling and ability to exploit grain legumes between storage and field environments (Utida, 1972; Sano-Fujii, 1984). *Callosobruchus maculatus* has remained a severe threat in cowpea-producing countries and is of great importance in Africa. Like other insect pests, the use of synthetic chemicals has been the primary means of control.

Insects rely on their sense of olfaction to locate their hosts (Bernays and Chapman, 1994). Progress made so far in the application of chemical ecology towards developing semiochemical-based control strategies has been on sex pheromones (Mullen, 1992; Phillips and Strand, 1994; Phillips, 1997; Scheirs *et al*., 2003) with a few on kairomones (Crook *et al*., 2008; El-Sayed *et al*., 2009; Nishida, 2014). The discoveries have led to the production of lures, an essential tool for monitoring insect pests (Phillips *et al*., 1993, 1996; Sharma and Fadamiro, 2013). However, we are unaware of any specific lure for monitoring *C. maculatus* or any study on host finding and the chemical cues that guide this pest to its preferred host plant in the field. Manipulation of host finding behaviour has been found more useful in pest management than manipulation of unrelated behaviours (such as mating) because it ensures an adequate protection of produce. Manipulation of non-pestilential behaviour may reduce the resident insect population but still not protect the produce from an influx of more insect pests (Cardè and Minks, 1995; Foster and Harris, 1997).

Based on earlier reports on the host choice of *C. maculatus* among legumes, coupled with its field-to-store route of infestation, we hypothesized that semiochemical cues from host legumes might play a significant role in the selection of a preferred host. The current study investigated possible cues for host finding in *C. maculatus* on three legume plants, namely Ife-brown (IB) and black-eyed (BE) cultivars of *V. unguiculata* and Pioneer 95Y70 cultivar of soybean (*Glycine max* (L.)) (SB), to determine factors responsible for preference of *C. maculatus* for the host legumes, the prerequisite to identifying chemical cues that mediate its host location. These three legumes were chosen because of their economic importance in Africa and beyond. Besides, IB has been found to be susceptible to *C. maculatus* and often used as a standard in cowpea–*C. maculatus* susceptibility tests in the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria (Mbata, 1993). We envisage that the results of this research will provide vital, necessary information on host location cues towards developing semiochemical-based pest management strategies for *C. maculatus*.

Materials and methods

Plant legumes

The two cultivars of cowpea, i.e. IB and BE, were obtained from African and grocery stores in Atlanta, GA and Auburn, AL, USA, respectively, while the Pioneer 95Y70 cultivar of SB was obtained from EV Smith Research farm in Shorter, AL, USA. Plant legumes were grown under modified greenhouse conditions (27 ± 2 °C and 55 ± 5% RH), as described by Balusu and Fadamiro (2011). Seeds were planted in pots in Sunshine potting mixture (Sunshine Mix #8), consisting of 70–80% Canadian sphagnum grower grade peat moss, coarse grade perlite, coarse grade vermiculite, dolomitic limestone (for pH adjustment), gypsum, and wetting agent (SunGro Horticulture, WA). Plants were irrigated once in 3 days and hydro-fertilized twice a week with Scotts Peat-Lite Special Fertilizer (Scotts-Sierra Horticultural Product Company, Marysville, OH, USA), a

20–10–20 water soluble N–P–K fertilizer mixed with micronutrients.

Insects

The culture of flight morph of *C. maculatus* started from a colony of sedentary morphs that had been maintained for over 100 generations in the insect rearing laboratory of the Department of Entomology and Plant Pathology, Auburn University, USA. Insects were reared as described by George and Verma (1999) with some modifications, taking into consideration all the factors inducing the development of flight morphs in *C. maculatus* as earlier reported (Utida, 1972; Sano-Fujii, 1979; Messina and Renwick, 1985). The beetles were sexed using the key described by Rees (2004). To obtain eggs that would develop to flight morph of *C. maculatus*, ~5-day-old beetles were used to start the culture (Sano-Fujii, 1979). Twenty-five pairs of the beetle were allowed ~48 h to lay eggs on 200 g cowpea seeds in 1 L wide-mouthed Ball® glass Mason jars. The cover of the jars consisted of nylon mesh of 1 mm × 1 mm aperture size held in place with a band on the lids. Insects were reared in a Percival Scientific incubator. The culture was started and maintained at 28 ± 2 °C, 70 ± 5% RH, and 12:12 h photoperiod for ~10 days. The conditions were adjusted to 35 ± 2 °C, 90 ± 5% RH, and 18:12 h photoperiod at ~11 days. To simulate a natural photoperiod, the inside of the incubator was modified to provide light and dark regime by using 122 cm, 40 watt fluorescent auto-tubes positioned at each corner of the incubator. Constant humidity was maintained by placing Petri dishes containing cotton wool saturated with water inside the incubator. Adults of flight morph of *C. maculatus* were identified using the features described by Utida (1972). The insects were used for the experiments starting from F_5 generation.

Glassware

The glassware used in behavioural bioassays and in the collection of volatile compounds were sterilized in a Glass Annealing Oven Model 120S, Volt 208, Hertz 60 (Wilt Industries Inc. RT 8, Lake Pleasant NY, 12108, USA) at 450 °C, to eliminate all organic stains or contaminants that may emit volatiles, thereby contaminating the required plant volatiles.

Behavioural bioassays

The olfactory response of mated ~3–4-day-old female flight morph of *C. maculatus* to legume plants was tested in two separate experiments using Y-tube and four-choice olfactometers. The Y-tube olfactometer bioassay was conducted to establish attraction of female beetles to each plant legume at green (PGP) and ripened pod (PRP) stages, and to compare attraction to plant without pod (PWP) with ripened pod (RP) under a no-choice condition. Four-choice olfactometer bioassays were conducted to determine the preference of *C. maculatus* among the three legume PGP and RP stages. Preference of the beetle between green pod (GP) and RPs of the three plant legumes was also determined. Attraction of the beetles to PWP, RPs, and GPs of the most attractive plant legume was compared.

Olfactometer bioassays were set-up as described by Morawo and Fadamiro (2014). The four-choice olfactometer consisted of a central chamber (30 cm long × 30 cm wide × 6 cm high) with orifices or ‘arms’ (17 cm long × 7 cm 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 glass chambers (22.8 cm diameter × 40.6 cm high) with lids, which housed the test plants. Each glass chamber had an inlet at the bottom and an outlet at the opposite top connected through Teflon tubing and ChemTred (8 mm i.d.) connectors to a flow meter on an air delivery system (ARS Inc., Gainesville, FL). This was, in turn, connected to an air source fitted with charcoal filter. The purified inlet air was pushed at a constant rate of 200 mL/min through the headspace of the test plants and removed by suction via a vacuum pump at the rate of 900 mL/min. The olfactometer apparatus was placed in a cardboard box (82 cm long × 82 cm wide × 61 cm high) lined with white paper and positioned under a fluorescent light source (~100 lux) for uniform lighting. An individual test plant was placed in a glass chamber. A replicate in four-choice tests consisted of a set of three host plants, and the control glass chamber that had no plant.

A Y-tube olfactometer (Analytical Research Systems, Gainesville, FL) was similarly connected except that it had two arms. One arm was connected to a host plant glass chamber and the second arm to a control (no plant). Air was removed by suction through the vacuum pump at the rate of 450 mL/min. Attraction of the beetles to the host plants in both olfactometers was tested in four replicates.

Mated female beetles (~3–4-day-old) were released individually into each olfactometer and allowed 10 min to make a choice among the olfactometer arms. In total, 30 female beetles were tested for each set of plant replicate. Beetles that failed to walk into any of the arms within 10 min were scored as ‘non-responders’, and were excluded in the analyses. Bioassays were conducted at 28 ± 2 º C, 60 ± 5% relative humidity.

Volatile organic compound (VOC) collection and GC analysis

Headspace volatile organic compounds (HS-VOCs) were collected from RP of IB and BE cultivars of cowpea, and SB. HS-VOCs were collected from 25 and 50 pods of cowpea and SB, respectively. The number of pods was chosen following the results of pilot tests conducted earlier. In total, 25 pods of cowpeas and 50 pods of SB produced consistent profiles in which key peaks were detected. The pods of each legume were placed in an airtight glass jar (5 L) (Analytical Research Systems, Inc., Gainesville, FL, USA). A purified (activated charcoal) stream of air at a constant rate of 500 mL/min was passed through the jar at room temperature for 24 h. Headspace volatiles were trapped with a trap containing 50 mg of Super-Q (Altech Associates, Deerfield, IL, USA) and eluted with 200 μL of methylene chloride. The resulting extracts (200 μL) were stored in a freezer (at −20 °C) until use.

Gas chromatography (GC) analysis was conducted to reveal key peaks of constituents of the HS-VOCs collected during pilot tests. As described by Ajayi *et al*. (2015), 1 µL of each headspace volatile 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., and 0.25 µm film thickness (Agilent Technologies Inc. Santa Clara, CA, USA). 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 held 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.

Gas chromatography–mass spectrophotometry analysis

Constituents of the HS-VOCs were identified by GC–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 μm 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 v2.0 using 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).

Statistical analysis

Data obtained in the Y-tube tests (attraction to individual plant legume) were analysed using a two-sided binomial test. Attraction of *C. maculatus* to the plant legumes in a four-choice olfactometer was modelled as a binary response count and treatments were compared using logistic regression analysis at 0.05% level of significance. The model adequacy for each set of experiments was confirmed with a likelihood ratio (Wajnberg and Haccou, 2008). Slopes were separated using Proc Logistic Contrast in SAS. For data presentation, *C. maculatus* attraction to plant legumes was represented on charts as percentage and count of beetles that responded in Y-tube and four-choice olfactometers, respectively. Data from GC-MS analysis were analysed by using a one-way ANOVA followed by the Tukey–Kramer HSD multiple comparison test at a significance level of $P < 0.05$.

Results

Attraction of flight morph of Callosobruchus maculatus *to plants of legume cultivars: Y-tube olfactometer bioassays*

Mated female flight morphs of *C. maculatus* were more attracted to the three plant legumes at green and RP stages compared to control (no plant) (Figs. 1 and 2). At the GP stage, significantly more: 93% (P = 0.000, binomial test), 87% (P = 0.000, binomial test), and 73% (P = 0.005, binomial test) *C. maculatus* chose IB, BE, and SB, respectively compared to control (no plant) (Fig. 1). PRP of IB, BE, and SB significantly attracted 100% (P = 0.000, binomial test), 90% (P = 0.000, binomial test), and 80% (P=0.001, binomial test) of beetles, respectively, compared to control (Fig. 2). Significantly, more beetles, 90% (P = 0.000, binomial test) and 83% (P = 0.000, binomial test) preferred RPs IB and BE, respectively, to PWP (Fig. 3). Attraction to RP (63%) and PWP (37%) of SB was not significant.

Responses of flight morph of Callosobruchus maculatus *to three legume plants: Four-choice olfactometer bioassays*

Female *C. maculatus* showed differential attraction to the three legume plants at all growth stages tested: PGPs ($\chi^2 = 155.62$, $df = 3$, $P < 0.0001$), plant with GP ($\chi^2 = 193.77$, $df = 3$, $P < 0.0001$), PRP ($\chi^2 = 177.45$, $df = 3$, $P < 0.000$), and plant with RP ($\chi^2 = 109.72$, $df = 3$, $P < 0.000$) (Fig. 4). Beetles failed to discriminate between SB and controls in PGP ($\chi^2 = 2.5435$, $df = 2$, $P = 0.1107$), GP ($\chi^2 = 0.3244$, $df = 2$, $P = 0.5689$), and PRP ($\chi^2 = 0.9216$, $df = 2$,

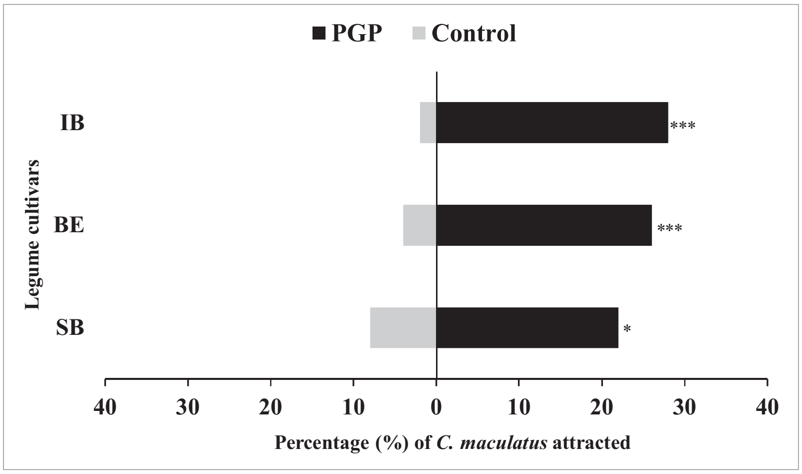

Fig. 1. Attraction of *Callosobruchus maculatus* adults in a Y-tube olfactometer to plants with green pods (PGP) of Ife brown (IB), black eyed (BE), and soybean (SB) compared to control (no plant) in three separate bioassays ($n = 30$). $*P < 0.05$, $**P < 0.001$, and $***P < 0.0001$ (two-sided binomial test; $\alpha = 0.05$).

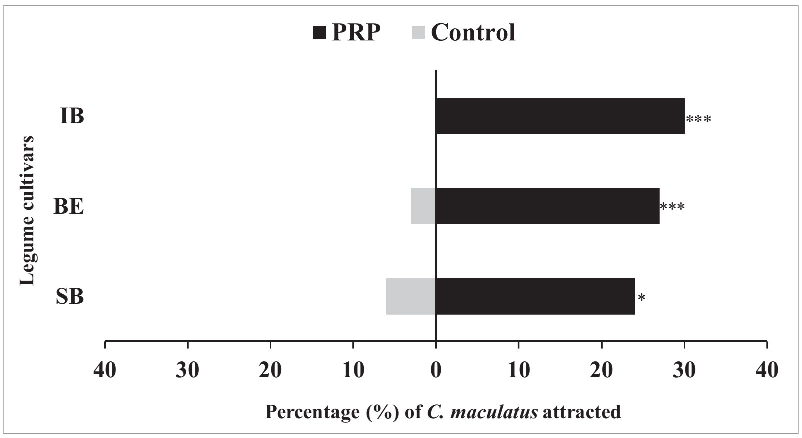

Fig. 2. Attraction of *Callosobruchus maculatus* adults in a Y-tube olfactometer to plants with ripened pods (PRP) of Ife brown (IB), black eyed (BE), and soybean (SB) compared to control (no plant) in three separate bioassays ($n = 30$). $*P < 0.05$, $**P < 0.001$, and $***P < 0.0001$ (two-sided binomial test; $\alpha = 0.05$).

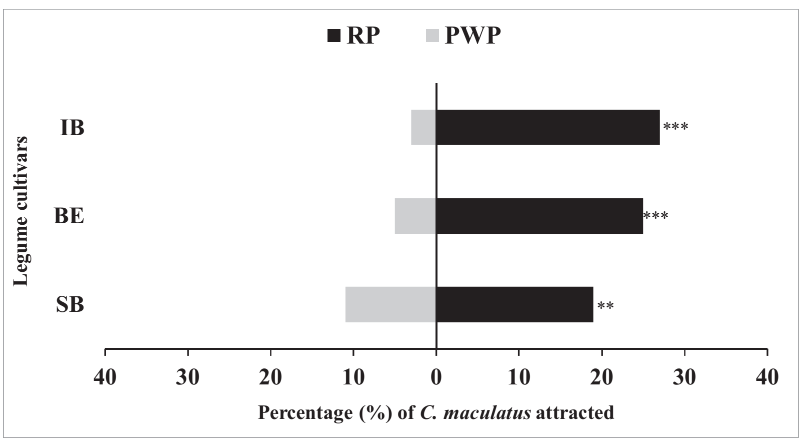

Fig. 3. Attraction of *Callosobruchus maculatus* adults in a Y-tube olfactometer to ripened pods (RP) of Ife brown (IB), black eyed (BE), and soybean (SB) compared to plants without pods (PWP) in three separate bioassays ($n = 30$). $**P < 0.001$ and $***P < 0.0001$ (two-sided binomial test; $\alpha = 0.05$).

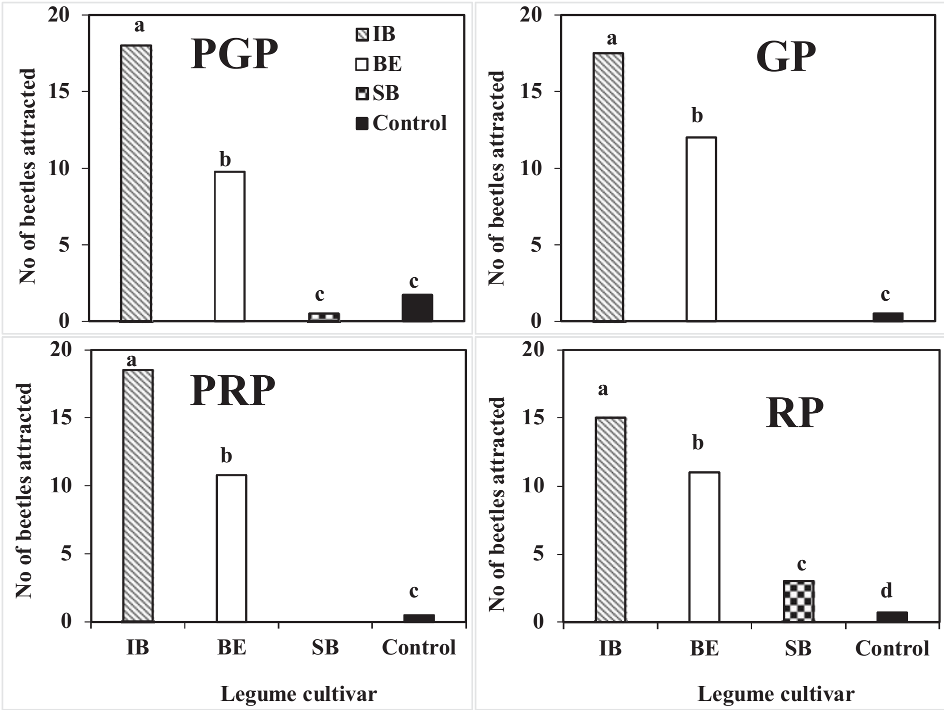

Fig. 4. Responses of *Callosobruchus maculatus* adults in a four-choice olfactometer to three legume cultivars: Ife brown (IB), black eyed (BE), and soybean (SB). PGP (plant with green pod), PRP (plant with ripened pods), GP (green pods only), and RP (ripened pods only) (n = 30). Bars with different letters are significantly different (Proc. logistic regression; $\alpha = 0.05$).

$P = 0.3371$) (Fig. 4), but showed preference for IB over BE cowpea in PGP ($\chi^2 = 19.9052$, $df = 2$, $P < 0.000$), GP ($\chi^2 = 38.6984$, $df = 2$, $P < 0.000$), PRP ($\chi^2 = 42.5351$, $df = 2$, $P < 0.0021$), and RP ($\chi^2 = 4.8573$, $df = 2$, $P < 0.0275$). Beetles showed differential attraction to phenological parts of IB cowpea ($\chi^2 = 95.45$, $df = 3$, $P < 0.000$) and were preferentially attracted to RPs (54%) compared to GPs (31.66%) and PWP (10.8%) (Fig. 5).

GC and GC–MS analyses

VOC profiles of the test legume cultivars varied qualitatively and quantitatively with five key peaks (compounds) detectable (Fig. 6). All the peaks (five compounds) were detected in IB, while BE cowpea and SB had three peaks and one peak, respectively. Two of these peaks were common in cowpea cultivars IB and BE. Only one of these peaks was detected consistently in the headspace of all the three legume cultivars. GC–MS revealed benzaldehyde in VOCs of IB and BE cowpea cultivars (Table 1). Emission of benzaldehyde was higher (8.23 ng/g of pod) in IB cowpea than in BE cowpea (6.32 ng/g of pod). One compound, octanone, was uniquely detected in BE cowpea. The only compound detected exclusively in SB was pentane.

Discussion

The three legumes, IB and BE cowpeas, and SB, plants had a significant attraction on *C. maculatus* in Y-tube (no choice) experiments, but in four-choice tests, the insect discriminated between the legume plants with preference to cowpea plants and a greater attraction to IB cowpea plant. Preference for cowpea plants confirms reports of earlier studies (Utida, 1972; Sano-Fujii, 1984; Messina and Renwick, 1985) that *C. maculatus* is a field-to-store pest of cowpea. Several studies list the broad spectrum of insect pests of SB in the field without a mention of *C. maculatus* (Singh, 1990; Kumar *et al*., 2012; Biswas, 2013). The close-to-zero attraction of *C. maculatus* to SB plant in four-choice tests supports the earlier reports that *C. maculatus* is not a field pest of SB, and that given a choice, it will not attack SB plant in the field. The active morph of *C. maculatus* attacks cowpeas in the field starting from pod forming (Zannou *et al*., 2003; Umar and Turaki, 2014) to ripened stage (Booker, 1967; Caswell, 1984; Zannou *et al*., 2003).

Increased attraction of beetles to cowpea plants containing pods compared with cowpea plants with no pod confirms earlier reports (Zannou *et al*., 2003; Umar and Turaki, 2014) and suggests that semiochemical cues emitted from pods possibly enhance host plant location in *C. maculatus*.

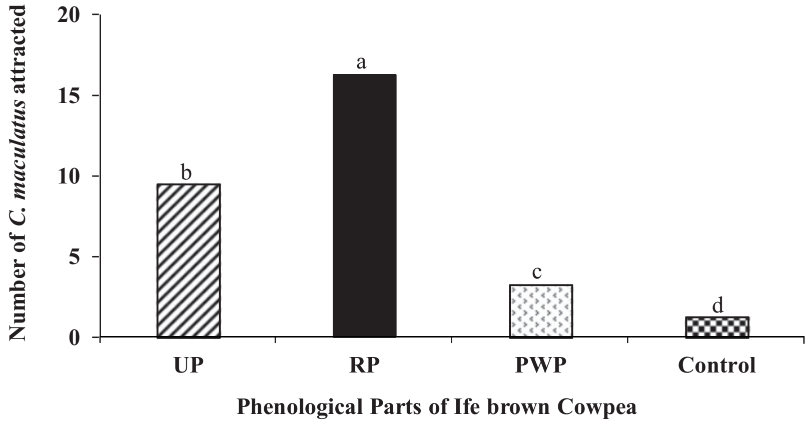

Fig. 5. Preferential attraction of *Callosobruchus maculatus* adults to three phenological structures of Ife-brown cowpea plant (UP = unripe pod, RP = ripened pod, and PWP = plant without pod) in a four-choice olfactometer (n = 30). Bars with different letters are significantly different (Proc. Logistic regression; $\alpha = 0.05$).

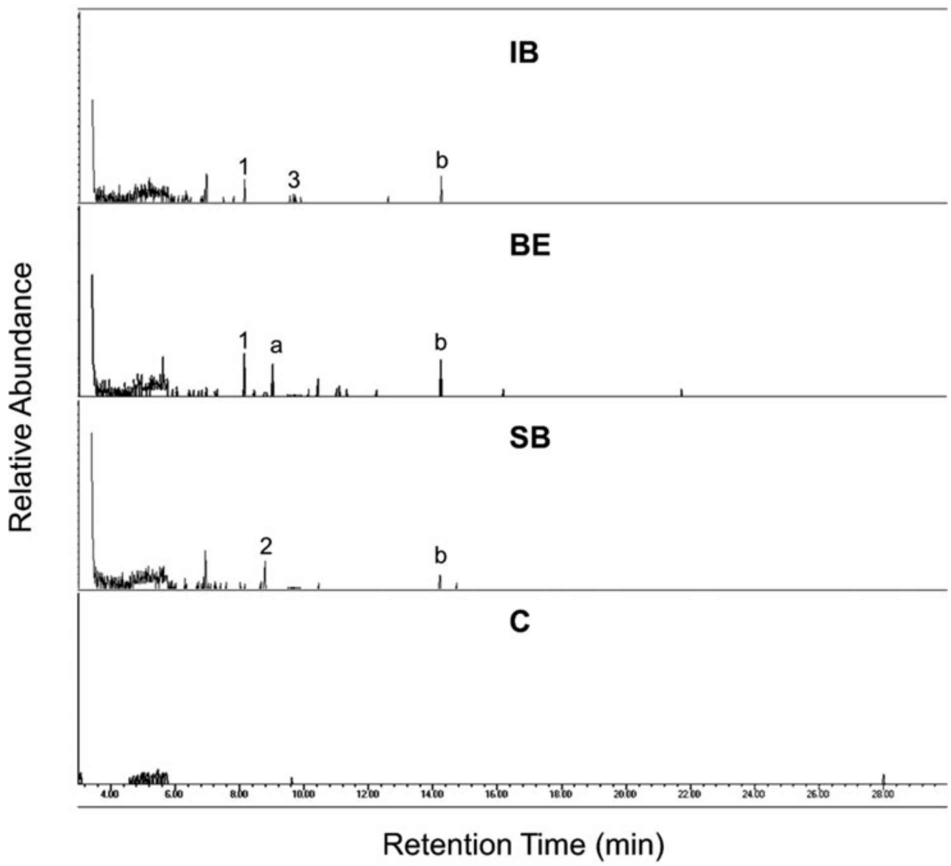

Fig. 6. Typical chromatographs showing components of headspace volatile organic compounds collected from ripened pods of IB (Ife brown), BE (black eyed), SB (soybean), and C (control).

Moreover, the ratio of compounds in host odour changes with phenological stage of different parts of the plant (Vallat and Dorn, 2005; Najar-Rodriguez *et al.*, 2010). Hence, the greater attraction of the beetle to RPs in IB cowpea compared to its GPs signalled possible biochemical changes in the VOCs of pods during ripening, which could result in the formation of distinct semiochemical cues. IB cowpea is more susceptible to insect pests than other cowpea cultivars; hence, it is used as a standard

Table 1. Quantities (mean ± SEM) (ng) of different components of headspace volatiles emitted by ripened pods of three legume cultivars

		Legume cultivars		
VOCs	RT (min)	Ife brown	Black eyed	Soybean
Benzaldehyde	8.188	8.23 ± 0.02b	6.32 ± 0.01a	ND
Pentane	8.772	ND	ND	8.13 ± 0.02
Unknown	8.902	ND	5.23 ± 0.02	ND
Octanone	9.012	6.27 ± 0.02	ND	ND
Unknown	14.012	11.35 ± 0.01a	11.24 ± 0.01a	11.17 ± 0.01a

Means within the same row having different letters are significantly different ($P < 0.05$).
VOCs, volatile organic compound; RT, retention time; ND, not detected.
Number of replicates per treatment = 3.

(negative control) in susceptibility tests in research (IITA, 1989; Mbata, 1993; Devereau *et al.*, 1999; Obadofin, 2014). In agreement with these earlier assertions and a previous study on legume seeds (Ajayi *et al.*, 2015), a higher number of *C. maculatus* consistently chose IB cowpea. Host location is one of the factors that determine insects' population build-up on host plants (Visser, 1986; Bruce *et al.*, 2005; Webster *et al.*, 2008). The odour of IB cowpea is possibly stronger than the odour of BE cowpea and SB. Previous studies (Ajayi *et al.*, 2015) and the present study reveal variations in semiochemical blends of the three legumes with the VOCs of IB having a more significant proportion quantitatively and qualitatively. It could be inferred from this study that the VOCs blend may likely be a factor in susceptibility of IB cowpea to insect pests as it will determine the level of its attraction to insect pests (IITA, 1989; Obadofin, 2014).

GC–MS revealed that VOCs of IB cowpea had a more significant quantity of biochemical components compared to the VOCs of BE cowpea and SB plants. Benzaldehyde was found in the volatiles of IB and BE cowpea. Earlier studies have confirmed attractivity of benzaldehyde to insects (James, 2005; Huber *et al.*, 2005; Najar-Rodriguez *et al.*, 2010; Kreuzwieser *et al.*, 2014). From the observed attraction of *C. maculatus* to BE cowpea plants in this study, benzaldehyde may likely be a primary cue in the attraction of the beetle to cowpea plants.

Genotype is an essential determinant of components of constitutive VOC in plants (Yuan *et al.* 2009). Variation in gene sequence among different cultivars or ecotypes within species can lead to a variation in chemical composition and products of physiology, and hence results in the diverse array of compounds in volatile emissions (Köllner *et al.*, 2004). Phenological parts of plants differ in chemical composition with a direct consequence on the quality and quantity of VOCs (Shiojiri and Karban, 2006; Li *et al.*, 2016), which may account for the differential attraction of the beetles to phenological parts of IB cowpea.

The differential attraction of *C. maculatus* to IB and BE cowpea may be a result of interspecific differences in the components of the VOCs of the two cowpea cultivars. Apart from the greater quantity of benzaldehyde in IB volatiles, octanone was found only in IB cowpea volatiles. Earlier studies confirm that octanone, a natural ketone, attracts insect species and it is a vital component of volatile blends that attract many fruit insects (Ashford, 1994; Yu *et al.*, 2011). Differences in biochemical compositions of VOCs of plants influence host recognition and acceptance in insects (Bruce *et al.*, 2005; Webster *et al.*, 2008). Studies have revealed that VOC blends are more attractive to insects than individual components (Hammack, 2001; Siderhurst and Jang, 2010; Beck *et al.*, 2012; Li *et al.*, 2012; McCormic *et al.*, 2012). Benzaldehyde and octanone are volatiles of agricultural importance, functioning in the attraction of pollinators (Birkett *et al.*, 2003; Huber *et al.*, 2005).

The ratio of compounds in volatile blends of plant species differs both qualitatively and quantitatively (Dötterl *et al.*, 2005; Jarriault *et al.*, 2009) even within the same genus as found in the case of IB and BE cowpea in this study. Variations in the ratio and combinations of compounds in blends of host volatiles are critical factors in host finding, recognition, and acceptance in insects (Visser, 1986; De Moraes *et al.*, 1998; Tasin *et al.*, 2006). Also, some compounds in a volatile blend could interact synergistically to elicit a greater behavioural or neurophysiological response in insects (Piñero and Dorn, 2007; D'Alessandro *et al.*, 2009). Hence, octanone (found only in the odour of IB cowpea plant), coupled with the greater attraction on beetles to IB compared with BE, signifies a possible synergistic role in the attraction of IB plant to *C. maculatus* in this study.

Conclusion

The results show the differential attraction of *C. maculatus* to the test legume plants. RPs were most attractive to *C. maculatus.* Infestation of cowpea plants in the field by *C. maculatus* could commence at GP stage, hence facilitating the need for proper monitoring. Further studies are ongoing to ascertain the attraction of flight morph of *C. maculatus* to benzaldehyde and octanone and the potential of these compounds as a lure for managing *C. maculatus* in cowpea farms.

Acknowledgements

This research was funded by Schlumberger Foundation, Faculty for the Future, through a fellowship to OEA. The authors thank Rammohan Balusu and Simon Zebelo for laboratory assistance. We also thank Dr Appel for allowing us to rear insects in his incubator.

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