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ORIGINAL PAPER

A major host plant volatile, 1-octen-3-ol, contributes to mating in the legume pod borer, *Maruca vitrata* (Fabricius) (Lepidoptera: Crambidae)

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Abstract Previous studies on the legume pod borer, *Maruca vitrata* Fabricius (Lepidoptera: Crambidae), a serious pest of cowpea, *Vigna unguiculata* (L.) Walp. (Fabales: Fabaceae), in sub-Saharan Africa have focused on sex pheromones, but the role of the host plant on sexual behavior has not been explored. We investigated this interaction in the laboratory using behavioral assays and chemical analyses. We found that the presence of cowpea seedlings and a dichloromethane extract of the leaf increased coupling in the legume pod borer by 33 and 61 %, respectively, compared to the control, suggesting the involvement of both contact and olfactory cues. We used coupled gas chromatography-electroantennographic detection (GC/EAD) and GC-mass spectrometry (GC/MS) to identify compounds from the cowpea leaf extract, detected by *M. vitrata* antenna. We found that the antennae of the insect consistently detected four components, with 1-octen-3-ol identified as a common and dominant component in both the volatiles released by the intact cowpea plant and leaf extract. We therefore investigated its role in the coupling of *M. vitrata*. In dose–response assays, 1-octen-3-ol increased coupling in *M. vitrata* with increasing dose of the compound compared to the control. Our results suggest that the cowpea volatile 1-octen-3-ol contributes to *M. vitrata* sexual behavior.

Keywords *Maruca vitrata* · Cowpea · Volatiles · 1-Octen-3-ol · Kairomone

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Introduction

Host plant volatiles are known to influence the behavior of phytophagous insects in various ways including their feeding (Gregory 1989), courtship and mating (Landolt and Phillips 1997), and finding suitable oviposition sites (Bruce et al. 2005). This is perhaps not surprising in nocturnal insects such as moths, which depend more on olfactory rather than visual cues for host location. Previous studies have also shown that plant volatiles can synergize sex pheromone production, release, and perception in some insects, typically in male moths (Landolt and Phillips 1997; Reddy and Guerrero 2004) such as *Spodoptera exigua* Hubner (Lepidoptera: Noctuidae) (Deng et al. 2004), *Cydia pomonella* Linnaeus (Lepidoptera: Tortricidae) (Yang et al. 2004), *Eupoecilia ambiguella* Hubner (Lepidoptera: Tortricidae) (Schmidt-Büsser et al. 2009), and *Lobesia botrana* Denis & Schiffermüller (Lepidoptera: Tortricidae) (von Arx et al. 2012). However, the involvement of plant volatiles in courtship and mating behavior of the legume pod borer (LPB) *Maruca vitrata* (Fabricius) (Lepidoptera: Crambidae), another nocturnal insect, has not been investigated.

The LPB, *M. vitrata*, is a serious pest of cowpea, *Vigna unguiculata* (L.) Walp. (Fabales: Fabaceae), an important food and forage legume in sub-Saharan Africa (SSA) and other tropical regions (Timko and Singh 2008). The most destructive stage of this insect is the larvae, which feed inside cowpea

flower buds, flowers, and young pods by first attacking reproductive parts of the flower (anthers, filaments, style, stigma, and ovaries). This typical feeding habit protects the larvae from natural enemies and other adverse factors including insecticides. Without control measures of the larval stage, a yield loss of up to 80 % has been reported in SSA (Sharma 1998). Control of *M. vitrata* heavily relies on the use of synthetic insecticides, of which its intensive use has led to environmental degradation, development of resistance to insecticide, killing of natural enemies, and high cost of applications (Adati et al. 2008). To reduce these adverse effects, an integrated pest management strategy such as the use of biopesticides and semiochemicals is advocated (Srinivasan 2012).

Previous studies have identified the sex pheromone components of *M. vitrata* from females. The first major pheromone component identified was (*E*, *E*)-10, 12-hexadecadienal (Adati and Tatsuki 1999), followed by additional components such as (*E*, *E*)-10, 12-hexadecadienol, (*E*)-10-hexadecenal (Downham et al. 2003), and (*E*)-10-hexadecenol (Hassan 2007) from the pheromone gland of the female. Several field evaluations of the pheromone blend, which were undertaken in different countries in SSA and South and Southeast Asia, gave variable results. In Benin, traps baited with a blend comprising (*E*, *E*)-10, 12-hexadecadienal, (*E*, *E*)-10,12-hexadecadienol, and (*E*)-10-hexadecenal in the ratio of 100:5:5 captured a significant number of male moths (Downham et al. 2004), while in Burkina Faso, Ghana, and Northern Nigeria, traps baited with (*E*, *E*)-10, 12-hexadecadienal alone was found to be effective. On the other hand, in India, a blend formulated from this component and (*E*)-10-hexadecenol proved to be more efficient in attracting males (Downham 2005, 2006; Hassan 2007), while in Taiwan, none of these pheromone blends attracted the moth (Schläger et al. 2012). While these observations point to the need for more research on the composition of the pheromone blend for its wide-scale use in the management of *M. vitrata*, it is also important to gain knowledge on the interaction between sexual communication in the moth and its host plant.

Field studies have shown that *M. vitrata* interaction with the cowpea plant can be categorized into three: damage at pre-flowering, flowering, and podding stages (Jackai 1981). Infestation starts in the terminal shoots before flowering (21 days after planting) then it later spreads to the reproductive parts. When the plant is in the reproductive stages, larval infestation takes the following order: flowers>flower buds>terminal shoots>pods (Sharma 1998). Furthermore, pheromone trap captures have shown that *M. vitrata* larval infestation preceded a week or more in advance of flowering in the cowpea fields (Downham 2006). Given these infestation patterns and pheromone trap captures, we hypothesized that adult moths arrive on the plants during the pre-flowering stage and the volatile cues released by this stage of the plant influence sexual responses in both sexes of the insect. In order to test this hypothesis, we investigated the influence of the major host plant (cowpea) on the mating behavior of *M. vitrata* in the laboratory with the following objectives: (1) to determine the effect of the presence of the cowpea plant on patterns of mating in order to define the time and age for optimal moth mating, (2) to assess the perception of the volatiles from the leaf extract and their effect on mating, and (3) to assess the influence of major antennally active component(s) in the volatiles of the leaf extract on mating of *M. vitrata*.

Materials and methods

Insects

Larval stages of *M. vitrata* were obtained from cowpea fields and were reared on semi-artificial diet as described by Onyango and Ochieng'-Odero (1993) at the *icipe's* Thomas Odhiambo Campus in Mbita, Kenya. They were shipped to the *icipe* Duduville, campus Nairobi as pupae (2 days old). The pupae were transferred to cages consisting of a cubic metal frame (30×30×30 cm) covered with a mosquito net of 1500 μm mesh size for adult emergence. The rearing room was maintained at 24±1 °C, relative humidity of 60±10 %, and a photoperiod of 12:12 (L/D). The moths emerged in about 5–7 days and were sexed with males and females kept separately for subsequent experiments. Sucrose solution (10 %), soaked in absorbent cotton wool, was provided as food and replaced after every 2 days.

Host plant

Cowpea plants, *Vigna unguiculata* (cv. Ex-Luanda), which is a local variety and more susceptible to *M. vitrata* than other common cowpea varieties, were used (Okeyo-Owuor et al. 1983). The plants were grown in sand-loamy soils in plots (4×4 m) within *icipe* Duduville, campus Nairobi.

Extraction of cowpea leaves

We harvested young and tender leaves (35 g) from 2-month-old cowpea plants at pre-flowering stage with a pair of forceps, and soaked them in 200 ml dichloromethane (Sigma-Aldrich, Chemie, Steinheim, Germany) without stirring for 30 min. This was replicated three times using a similar weight of tender leaves harvested from different plants. The residue from each extraction was filtered off and the extract dried over anhydrous $MgSO_4$ (Sigma-Aldrich, Chemie, Steinheim, Germany), before being concentrated to 1 ml in vacuo at 40 °C at atmospheric pressure using a rotary evaporator at a speed of 30 rpm (Laborota 4000 efficient, Heizbad HB digit; Heidolph Instruments, Germany).

Mating bioassays in the presence of host plant and leaf extract

Our preliminary observations showed that coupling in *M. vitrata* occurred during the scotophase for about 15–30 min, and males make several mating attempts with different females before successfully coupling with females. Bioassays were therefore conducted at night, and they involved two treatments and a control, each consisting of 20 pairs of newly emerged males and females (1 day old). This was carried out in Perspex cages (20×20×20 cm) and sucrose solution (10 %) was provided as food, replaced after every 2 days. Treatment 1 consisted of pairs of moths in the presence of four potted cowpea seedlings (2 weeks old). Treatment 2 had pairs of moths in cages containing a filter paper disc (1.5 cm diameter; Macherey-Nagel MN 615) loaded with different doses of cowpea leaf extract; 12.5 μg/disc and 125 μg/disc, equivalent to 1 and 10 plant hours (i.e., the amount of 1-octen-3-ol released per hour at night by 1 and 10 cowpea seedlings of the same age, respectively, based on our preliminary observations). The control in both assays was similar pairs of moths alone. During each bioassay with newly emerged *M. vitrata*, we recorded the number of mating pairs, irrespective of whether multiple mating occurred or not, at hourly intervals between 18:00 and 06:00 h each day for 7 days. This approach enabled us to define the time and age for optimal mating in *M. vitrata* compared to the control. All the bioassays were carried out under a 9-W red lamp (230 V—50 Hz), which had no effect on mating of the moth (Hassan 2007). In all the experiments, a total of 60 pairs of moths were used comprising three replicates for each treatment.

Collection of volatiles from cowpea plant

We trapped headspace volatiles from cowpea plants grown in the field at the *icipe*, Duduville campus for 11 h each during the night (19:00–06:00 h) using a portable battery-powered pump (assembled at the USDA/ARS-CMAVE, Gainesville, FL, USA). Transparent oven bags (Baco and BacoFoil; Wrap Film Systems Ltd., UK) (25×38 cm) were pre-conditioned at 150 °C for 12 h. We enclosed one cowpea plant with the pre-cleaned oven bag and passed charcoal filtered air through it at 250 ml/min into Super-Q traps (30 mg; Alltech, Nicholasville, KY). A blank oven bag trapped for the same period was used as a control. The Super-Q traps were eluted with 150 μl of dichloromethane (Sigma-Aldrich, Chemie, Steinheim, Germany) and the eluents stored at −80 °C until used. We collected volatiles from pre-flowering plants during the night and utilized the same plant stage as in the mating bioassays. Three replicates were used with a different plant in each replicate.

Collection of volatiles from leaf extract

To determine the rate of emission of the volatile components from the leaf extract, we applied 100 μl containing 125 μg of the extract on to filter paper discs as described above under the "Mating bioassays in the presence of host plant and leaf extract" section. The filter paper disc (1.5 cm diameter) was placed in an open glass vial (30 ml) to simulate their use in the mating bioassays carried out previously (see "Mating bioassays in the presence of host plant and leaf extract"). We used solid phase micro-extraction (SPME) technique to capture volatiles on a 65-μm polydimethylsiloxane-divinylbenzene (PDMS/DVB) (Supelco, Bellefonte, PA, USA) fiber and then analyzed the volatiles captured on the fiber directly by gas chromatography coupled to a mass spectrometer (GC/MS) (conditions as described in the "Statistical analysis" section). Volatiles were sampled continuously from the filter paper loaded with leaf extract using separate SPME fibers, which were exposed to the extract for an hour each time for a total of 12 h (same time used for mating bioassays). Prior to use, we preconditioned the fibers at 250 °C for 30 min in the GC. After sampling, the SPME fiber was inserted into the injector port of a gas chromatograph fitted with a 4.0-mm gooseneck splitless, taper liner (Agilent, Palo Alto, California, USA) for a 2-min desorption period at 250 °C. After injection, each fiber was cleaned as above, retracted into its holder, and stored for further sampling. Three replicates were used in this experiment. We identified the electroantennographic-active (EAG-active) components by comparing their mass spectral data with library data, Adams2 (Adams 1995) and National Institutes of Standards and Technology (2005), and retention times with those of authentic compounds.

Analyses of cowpea volatiles and leaf extract

We analyzed the cowpea leaf extract using coupled gas chromatography/electroantennographic detector (GC/EAD) on a Hewlett-Packard (HP) 5890 Series II gas chromatograph equipped with a HP-5MS column (30 m×0.25 mm ID×0.25 μm; Agilent, Palo Alto, California, USA) with nitrogen as the carrier gas. The extracts were analyzed in a split/splitless mode at an injector temperature of 280 °C and a split valve delay of 0.8 min. The oven temperature was held at 35 °C for 3 min, then programmed at 10 °C/min to 280 °C and maintained at this temperature for 10 min. The column effluent was split 1:1 for simultaneous detection by flame ionization detector (FID) and EAD. Silver-coated wires in drawn-out glass capillaries (1.5 mm ID) filled with Ringer saline solution (Kugel 1977) served as reference and recording electrodes in the EAD detection. Antenna (4–5-day-old adult moth, optimum age for mating; see "Results" section) was prepared by cutting

the base and distal ends with a scalpel blade. The reference electrode was connected to the base of the antenna, and the recording electrode was connected to the cut tip of the antenna. The analog signal was detected through a probe (INR-II; Syntech, Hilversum, the Netherlands), captured and processed with a data acquisition controller (IDAC-4; Syntech, the Netherlands), and later analyzed with a software (EAG 2000; Syntech) on a personal computer. An aliquot (5 μl) of the leaf extract was analyzed with antennae of both male and female. This was replicated three times using fresh antennae.

For identification, both the leaf extracts and volatiles trapped on SPME fibers and Super-Q (see volatile collection) were analyzed using a split/splitless injection on an Agilent technologies-7890 gas chromatograph coupled to 5975C inert XL EI/CI mass spectrometer (EI, 70 eV; Agilent, Palo Alto, California, USA) (GC/MS) equipped with an HP-5MS column (30 m×0.250 mm ID×0.25 μm; Agilent, Palo Alto, California, USA). The injector temperature was 280 °C with a split valve delay of 0.8 min. Helium was used as the carrier gas at a flow rate of 1.25 ml/min employing the oven conditions described above. The EAG-active components were identified by comparing their mass spectral data with library data, Adams2 (Adams 1995) and National Institutes of Standards and Technology (NIST 2005), and retention times with those of authentic compounds.

Mating bioassays in the presence of 1-octen-3-ol

1-Octen-3-ol, the major and consistent antennally detected cowpea volatile identified in this study (see "Results" section), was tested in a dose–response assay as follows: we loaded filter paper discs (1.5 cm diameter; Macherey-Nagel MN 615) with 1, 2, and 3 μg of 1-octen-3-ol dissolved in dichloromethane and tested these in three individual cages each consisting 20 pairs of moths. The fourth cage with moths alone served as a control. The setup was as described in the previous section in the Perspex cages under same conditions. Each dose tested was replicated three times with 60 pairs of moths per treatment.

Chemicals

1-Octen-3-ol (98 % racemic mixture) and (*E*)-2-hexenal (98 %) were purchased from Aldrich Chemical Company Inc., USA. (*Z*)-3-Hexen-1-ol (98 %) was obtained from Fluka Chemie, Sigma-Aldrich, Switzerland.

Statistical analysis

We used a multivariable Poisson regression model to study the association between the number of mating moth pairs (n=60) and treatments. The model was fitted with the logarithm of the total number of pairs per hour and per day introduced as an offset. We estimated the risk ratios (RR) for treatments in comparison with the control and with reference to the optimum time and age in days. The RR measured the effect of the treatments on the mating behavior of the moths. It gives the probability of finding couples mating under a given treatment at a particular time with reference to a control. R version 2.15.1 statistical package was used for data analyses (R Development Team 2012).

Results

Mating bioassays in the presence of host plant and leaf extract

We found that while cowpea seedlings increased mating in *M. vitrata* by 33 % (RR=1.33, 95 % CI 0.99–1.79, P=0.0611), the leaf extract (125 μg/disc) increased mating significantly by 61 % (RR=1.61, 95 % CI 1.21–2.15, P<0.01) compared to the control (Fig. 1 and Online resource, Table OR1). We found significant differences in the number of mating pairs in treatment 1 (cowpea seedlings) and treatment 2 (leaf extract, 125 μg/disc) compared to the control at age 4 (P<0.01). Interestingly, a significant increase in the number of mating pairs also occurred at age 6 only on the leaf extract (125 μg/disc) compared to the control (Fig. 1c), but not in the presence of cowpea seedlings (Fig. 1a). We also found significant overall mating time effect for all the treatments used, with optimal mating time being at 02:00–03:00 h (P<0.001) (Fig. 1). However, there was no significant difference between the number of mating pairs found at 02:00 and 03:00 h, and also at 01:00 h for both treatments 1 and 2 (Fig. 1 and Online resource Table OR1).

Analyses of leaf extract

Using male and female antennae, four GC/EAD-active components from the leaf extract were located. These components were identified by GC/MS, namely, (*Z*)-3-hexenal, (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, and 1-octen-3-ol (Fig. 2). Of these compounds, except for (*Z*)-3-hexenal whose identity was tentatively established based on library data only since its commercial standard was unavailable to confirm the identification, the identities of the other three compounds were confirmed with commercial standards by both GC/EAD and GC/MS analyses. Generally, antennae of males elicited stronger EAD activity to 1-octen-3-ol and (*Z*)-3-hexen-1-ol in the natural extract (Fig. 2) and synthetic compounds (Fig. 3) than females. 1-Octen-3-ol was also the most abundant component (~88 %) when we collected headspace volatiles from the dichloromethane extract of cowpea leaves applied on to filter paper discs. The relative abundances of the other three components, (*Z*)-3-hexenal, (*E*)-2-hexenal, and (*Z*)-3-hexen-1-ol,

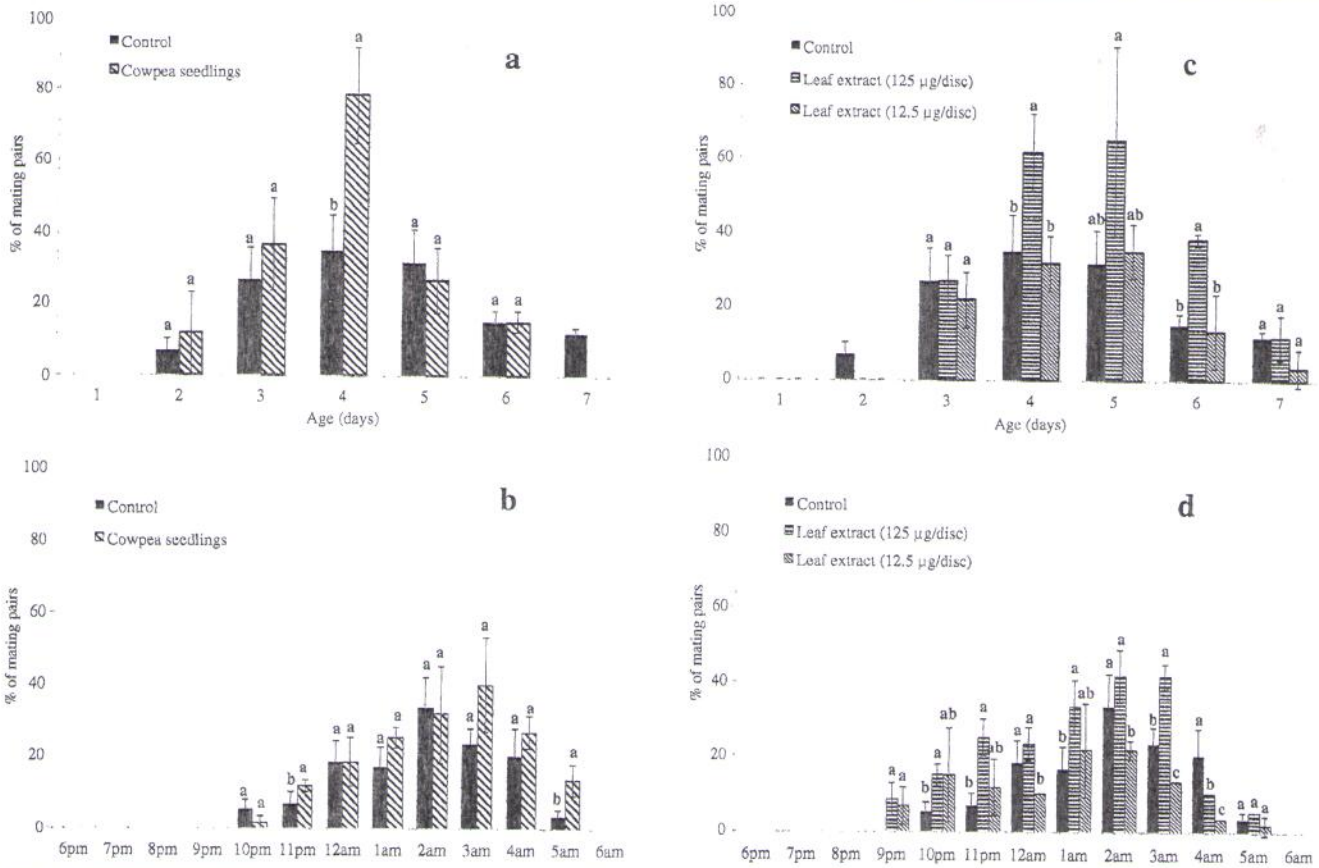

Fig. 1 Percentage of mating pairs in the presence of cowpea seedlings and cowpea leaf extract compared to control with respect to age in days (**a**, **c**) and time (**b**, **d**). *Bars* followed by different *letters* within each age group and time of the day are significantly different at $P<0.01$; number of insects used in the treatment, $n=60$

were ~2, 3, and 7 %, respectively. This is illustrated in our SPME analysis of the volatiles released from the filter paper loaded with 125 µg of the leaf extract, where 1-octen-3-ol dominated the volatiles throughout the 12-h sampling period (Online resource Fig. OR1). The relative amount of 1-octen-3-ol in the headspace volatiles of the cowpea plant in the pre-flowering stage at night (the time when bioassays were carried out) was also higher than the amounts of other EAD-active components identified (Fig. 4).

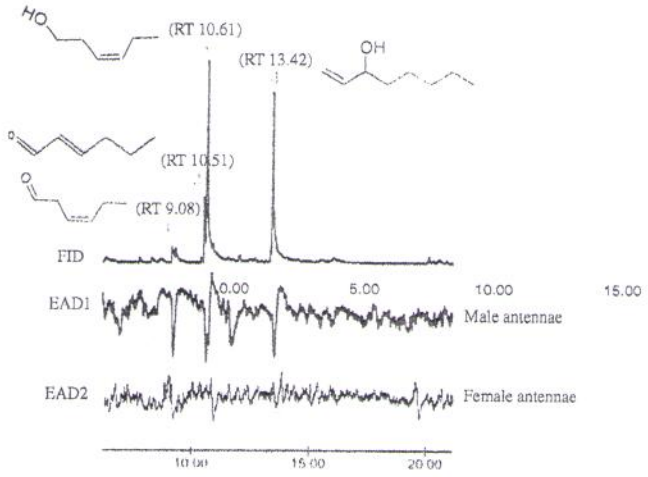

Fig. 2 Representative GC/EAD profile using both male and female antennae of *M. vitrata* to compounds in the cowpea leaf extract. The EAD-active compounds include (*Z*)-3-hexenal (*1*), (*E*)-2-hexenal (*2*), (*Z*)-3-hexenol (*3*), and 1-octen-3-ol (*4*)

Mating bioassays in the presence of 1-octen-3-ol

In dose–response assays with 1-octen-3-ol, significant numbers of mating pairs were recorded at 1 µg/disc (RR=1.51, 95 % CI 1.06–2.16, $P<0.05$) and 2 µg/disc (RR=1.59, 95 % CI 1.12–2.27, $P<0.01$), with the most stimulatory dose being 3 µg/disc (RR=2.25, 95 % CI 1.63–3.16, $P<0.001$) over the control (Fig. 5 and Online resource, Table OR2). A significant difference was observed among the doses ($\chi^2=24.7$, $df=3$, $P<0.001$). The number of mating pairs peaked at day 4 (Fig. 5), and significant differences were observed within the three doses ($\chi^2=13.7$, $df=3$, $P<0.01$) (Online resource, Table OR2). The overall treatment effect in the number of mating pairs was similar among the three treatments, viz., 1-octen-3-ol (3 µg/disc), leaf extract, and cowpea seedlings ($\chi^2=2.0$, $df=2$, $P=0.36$), with the optimal age for mating occurring at day 4 ($\chi^2=1.7$, $df=2$, $P=0.42$) (Online resource, Table OR3).

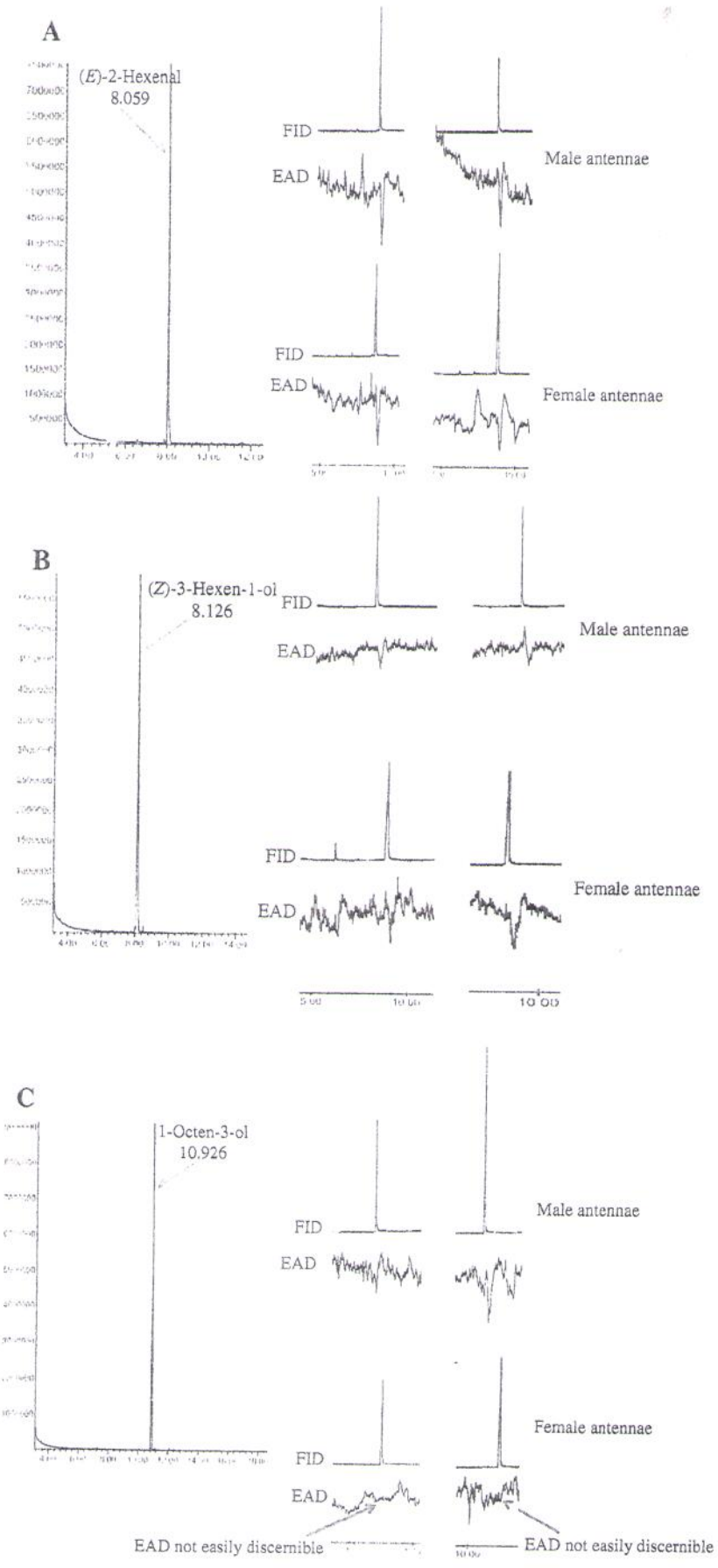

Fig. 3 Representative GC/MS and GC/EAD profile using both male and female antennae of *M. vitrata* to selected authentic standards of EAD-active compounds; (*E*)-2-hexenal (**a**), (*Z*)-3-hexenol (**b**), 1-octen-3-ol (**c**)

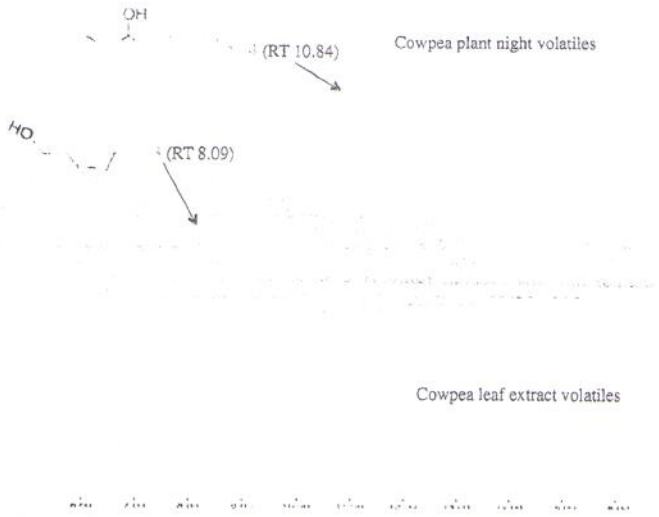

Fig. 4 Mirrored GC/MS profile of cowpea plant night volatiles and cowpea leaf extract volatiles showing two EAD-active components, (*Z*)-3-hexenol (*3*) and 1-octen-3-ol (*4*)

Discussion

Sexual communication in phytophagous insects is strongly influenced by host plant volatiles (Raina et al. 1992; Landolt and Phillips 1997), which are known to act on the nervous and hormonal systems of female moths to stimulate pheromone production and release, and also to enhance attraction of male moths to female-produced sex pheromones (Reddy and Guerrero 2004). Our results which showed a significant number of mating pairs in the presence of the host plant suggest an increased calling from females in response to host plant odors, leading to a stronger male response to female odors. Our results also suggest that host plant odors play a role in male response to female calling behavior. Several studies have shown that host plant odors trigger increased calling response from female moths (McNeil and Delisle 1989a; Reddy and Guerrero 2004). For example, the presence of pollen or an ethanolic pollen extract from the sunflower, *Helianthus annuus* (Asterales: Asteraceae), a host plant of the sunflower moth, *Homoeosoma electellum* Hulst (Lepidoptera: Pyralidae), initiated calling behavior in females at a younger age than similar-aged females provided with sucrose only (McNeil and Delisle 1989b). Sex pheromone release in the cabbage looper moth, *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae), is also stimulated in the presence of the host plant (Landolt et al. 1994). Our finding that the presence of the host plant recorded the maximum number of mating couples on days 4 and 5 and at between 02:00 and 03:00 h is in agreement with the mating pattern observed in a previous study carried out in Nigeria by Jackai et al. (1990). In contrast, a similar study carried out in Taiwan (Huang and Peng 2001) and China (Lu et al. 2007) recorded the maximum number of mating pairs on day 3, at 24:00 and 05:30 h and at 23:00 and 24:00 h, respectively. Experimental and rearing conditions for the moth could account for the differences in results. Adati et al. (2004) reported that temperature influenced the development of all the stages and the sexual maturity of *M. vitrata*. In our study, the rearing temperature was 24±1 °C, whereas Jackai et al. (1990) used a temperature range of 20–25 °C. On the other hand, Huang and Peng (2001) working in Taiwan and Lu et al. 2007 in China maintained their colonies at slightly higher temperatures: 27±1 and 29±1 °C, respectively. Larval diet has also been shown to contribute to how long it takes for the LPB to develop to the adult stage (Kudo et al. 2014). While we used semi-artificial diet for rearing comprising mainly cowpea flower powder and soybean flour (Onyango and Ochieng'-Odero 1993), in the Taiwan study, larvae were reared on the legume, *Sesbania cannabina* Retz. (Fabales: Leguminosae) (Huang and Peng 2001), with cowpea flowers used in the China study (Lu et al. 2007).

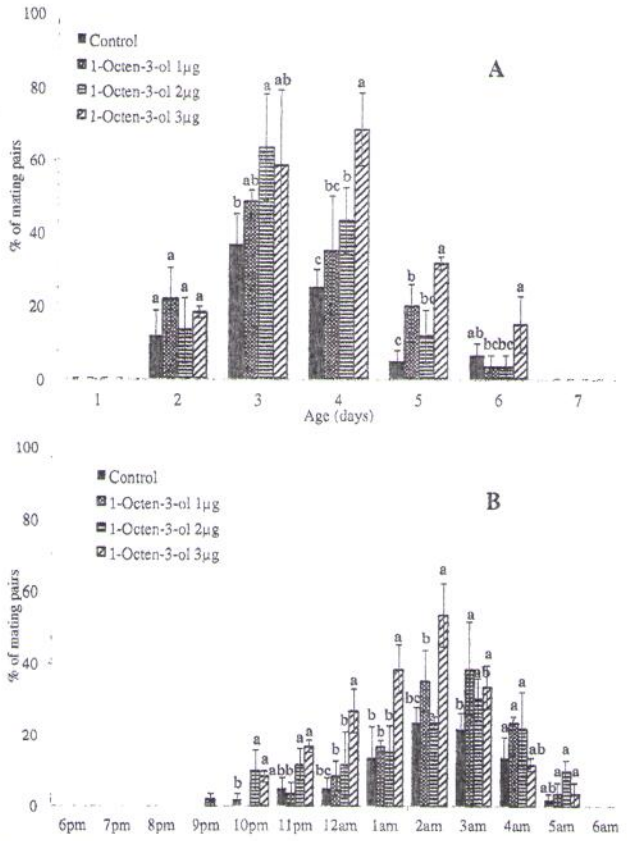

Fig. 5 Percentage of mating pairs in the presence of 1-octen-3-ol at 1, 2, and 3 µg/disc compared to control with respect to time and age in days. *Bars* followed by different *letters* within each age group and time of the day are significantly different at $P < 0.01$; number of insects used in the treatment, $n = 60$

Regarding the assays on the leaf extract, the enhanced coupling response exhibited by *M. vitrata* to the leaf extract odors over the intact plant strongly suggests that olfactory rather than visual cues may play a more significant role in host location in *M. vitrata*. There are a number of reasons that could have contributed to the difference between the mating

response of the moth in the presence of the plant and the leaf extract. For example, the presence of the plant provides visual cues that may trigger oviposition and feeding in the different sexes thereby interfering with sexual responses. The plant's physical structure may also interfere with mating by serving as a mechanical barrier and a platform for resting or congregation of the insects. Additional reasons include possible differences in the quality and quantity and release rates of the volatiles emitted from the intact plant and leaf extract loaded on to filter paper and captured on the different adsorbents (SPME fiber and Super-Q) we used. Therefore, the volatile profile of the leaf extract may represent a snapshot of what was released by the leaf at the moment when it was extracted whereas the Super-Q collected volatiles may represent volatile organic compounds produced by the plant metabolism over a number of hours. We also observed that coupling in the moth extended to day 6 in the presence of both the plant and olfactory cues, but this behavioral response was stronger in the latter alone, suggesting the important role played by cowpea volatiles in influencing mating in *M. vitrata*.

Our GC/EAD and GC/MS analyses identified four EAD-active components in the leaf extract, derived from two classes: alcohols ((*Z*)-3-hexen-1-ol and 1-octen-3-ol) and aldehydes ((*Z*)-3-hexenal and (*E*)-2-hexenal). The C_6 derivatives are "green leaf volatiles" released by plants after tissue damage (Ngumbi et al. 2005; Dicke and Baldwin 2010; Fürstenau et al. 2012; Tang et al. 2012; Allmann et al. 2013). As such, in the headspace, the low levels of (*Z*)-3-hexen-1-ol, (*Z*)-3-hexenal, and (*E*)-2-hexenal detected in the night time volatiles may indicate leaf tissue damage due to handling. However, this does not rule out the possibility that they may play a role as synergists or additives in the overall mating response of *M. vitrata*. Indeed, previous studies have reported that some of these leaf tissue damage volatiles combined with sex pheromones influence sex communication in lepidopterans. For example, traps baited with (*E*)-2-hexenal combined with the sex pheromone lure, (*Z*,*E*)-9,12-tetradecadienyl acetate, (*Z*,*E*)-9,12-tetradecadienol, and (*Z*)-9-tetradecenyl acetate increased captures of *Spodoptera exigua* Hubner (Lepidoptera: Noctuidae) by 38 % compared to traps baited with the pheromone alone (Deng et al. 2004). Similar findings were obtained for the diamondback moth, *Plutella xylostella* Linnaeus (Lepidoptera: Plutellidae), when (*Z*)-3-hexen-1-ol and (*Z*)-3-hexenyl acetate were combined with the sex pheromone lure, (*Z*)-11-hexadecenal, (*Z*)-11-hexadecenyl acetate, and (Z)-11-hexadecenal (Dai et al. 2008). Of the four EAD-active components, 1-octen-3-ol was consistently identified as the dominant volatile by GC/MS in both the headspace volatiles of the intact plant and that captured by SPME from the dichloromethane cowpea leaf extract loaded on to filter paper. We therefore tested it for behavioral activity.

Our bioassay with 1-octen-3-ol revealed a dose-dependent relationship, which compared favorably to that of the leaf extract and cowpea seedlings, with the optimal mating age of the moth being 4 days old. This demonstrated that this component contributes to *M. vitrata* sexual behavior. Interestingly, 1-octen-3-ol is a chiral compound with only two enantiomers, (*S*)-(+)-1-octen-3-ol and (*R*)-(−)-1-octen-3-ol, and it is likely that the cowpea plant produces only one enantiomer. Thus, our results suggest that the moth's detection of the compound is not antagonized by the unnatural enantiomer. Behavioral activity has been reported in previous studies for 1-octen-3-ol in different insects. For example, in grapevine and grape berry, it is a key kairomonal component that increases activation and attraction of males of the European grapevine moth *Lobesia botrana* Denis & Schiffermüller (Lepidoptera: Tortricidae) and the European grape berry moth *Eupocilia ambiguella* Hubner (Lepidoptera: Tortricidae) to their sex pheromones (Schmidt-Büsser et al. 2009; von Arx et al. 2011, 2012). It has also been found to elicit upwind flight in mated females of the grapevine moth (Tasin et al. 2006) and to attract a significant number of male and female navel orange worm moths, *Amyelois transitella* Walker (Lepidoptera: Pyralidae), in almond orchards (Beck et al. 2012). Given our findings, the assessment of volatiles associated with other leguminous host plants attacked by the moth such as pigeon pea, mung bean, and yard-long beans on the sexual behavior of *M. vitrata* could provide additional useful information about the chemical ecology of this insect towards its management.

In summary, this work has demonstrated for the first time that cowpea volatiles play a key role in the mating behavior of *M. vitrata* and that they can possibly augment sex responses in both sexes. Future research will focus on testing male responses to 1-octen-3-ol combined with tissue damage volatiles and also the synthetic components of the female-produced sex pheromone of the insect.

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