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Ability of bed bug (Hemiptera: Cimicidae) defensive secretions (*E*)-2-hexenal and (*E*)-2-octenal to attract adults of the common bed bug *Cimex lectularius*

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Abstract. Accurate and timely surveillance of bed bug infestations is critical for the development of effective control strategies. Although the bed bug-produced volatiles (*E*)-2-hexenal and (*E*)-2-octenal are considered as defensive secretions, the present study demonstrates, using ETHOVISION® video-tracking software (Noldus Information Technology Inc., Leesburg, Virginia), that low amounts of these commercially-obtained aldehydes function as attractants, and high amounts function as local repellents, against the common bed bug *Cimex lectularius* L. In behavioural assays, both males and female *C. lectularius* are attracted to 0.04 μg of an aldehyde blend (1 : 1) for up to 1 h after initial treatment of filter paper disks. Males differ from females in their response to higher amounts of aldehydes, with females and males exhibiting maximum local repellency at 40 μg and 400 μg, respectively. The results suggest that these bed bug secretions may be candidates for lures and monitors.

Key words. Aldehydes, attraction, *Cimex lectularius*, defensive secretions.

Introduction

The common bed bug, *Cimex lectularius* L., is a nocturnal ectoparasite of humans that feeds solely on blood. The pest's cryptic nature makes it difficult to detect infestations, and renders eradication both challenging and expensive because commercial treatments can be prohibitively costly. Do-it-yourself products such as foggers are ineffective (Jones & Bryant, 2012) and bed bug-related insecticide misuse has been documented to cause illnesses, and at least one death has been associated with insecticide misuse (Centers for Disease Control and Prevention, 2011). Failure to treat infestations quickly and adequately enables bed bugs to spread to new areas of a home or building, thereby exacerbating control efforts (Wang *et al.*, 2010). For this reason, the early detection of bed bug populations is critical.

Effective tools and methods of augmenting visual inspections are valuable for bed bug detection. For example, canines can be trained to alert to bed bugs (Pfiester *et al.*, 2008; Vaidyanathan & Feldlaufer, 2013), although a lack of standardized canine training coupled with handler inexperience can affect the accuracy of detection (Cooper *et al.*, 2014). Various bed bug 'monitors' have also been developed that operate either without a lure or bait (passive) or with an attractant, such as heat, carbon dioxide or other host-specific cues (active) (Vaidyanathan & Feldlaufer, 2013). Descriptions are available for active monitors using either host-specific cues, such as carbon dioxide, octenol and lactic acid (Wang *et al.*, 2011; Singh *et al.*, 2012; Aak *et al.*, 2014), or bed bug-derived components from scent glands and faecal excrement (Siljander *et al.*, 2008; Olson *et al.*, 2009; Weeks *et al.*, 2013). Although numerous monitors have been developed, no type (passive or active) has gained widespread use, primarily because of inconsistencies under field conditions (Weeks *et al.*, 2011a).

Because both the common bed bug and the tropical bed bug (*C. hemipterus*) aggregate, presumably mediated by bed bug-produced or bed bug-associated chemical cues (Marx, 1955; Siljander *et al.*, 2007, 2008; Weeks *et al.*, 2011b; Mendki *et al.*, 2014), bed bug aggregation has been studied in an attempt to develop a monitor or lure. Interestingly, two bed bug secretions, (*E*)-2-hexenal and (*E*)-2-octenal, are reported from *C. lectularius* as compounds that elicit both an alerting and aggregating

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behaviour (Levinson & Bar Ilan, 1971; Gries *et al.*, 2014). The metathoracic scent glands of adults produce predominantly aldehydes (Schildknecht *et al.*, 1964), with (*E*)-2-hexenal being produced at a three-fold rate higher than the other main aldehyde, (*E*)-2-octenal (Collins, 1968; Levinson & Bar Ilan, 1971). However, in *C. lectularius* nymphs, (*E*)-2-octenal is three-fold more abundant than (*E*)-2-hexenal (Levinson *et al.*, 1974). At high concentrations, these aldehydes apparently induce dispersal and alarm responses (Levinson & Bar Ilan, 1971). The same aldehydes, along with associated 4-oxo-aldehydes (Feldlaufer *et al.*, 2010), also play a role in predator avoidance and defence, as well as in conspecific cues with respect to mating (Ryne, 2009; Harraca *et al.*, 2010).

The accurate and precise surveillance of bed bug infestation is an essential first step in the development of a management strategy. To be commercially viable, a lure must attract bed bugs regardless of sex or life stage, be persistent, and require minimal care and follow-up from a technician or applicator between visits. Although several compounds have been identified and tested in laboratory and field conditions, there are gaps in our understanding of how bed bugs behave and interact with aldehydes at different concentrations. To investigate the time frame of attractiveness of C. *lectularius* volatiles, the movement of adult bed bugs in response varying amounts of aldehyde blends is examined. Doses of bed bug volatiles that elicite avoidance behaviour, as would be expected of an alarm or dispersal pheromone, are also determined.

Materials and methods

Insects

A colony of *C. lectularius* was established from bed bugs originally obtained from Harold Harlan (Crownsville, Maryland). The colony was reared at ambient laboratory conditions ($25 \pm 2\,^{\circ}C$ and $40 \pm 15\%$ relative humidity) and fed weekly on expired, human red blood cells fortified with plasma, using an artificial (*in vitro*) feeding system (Feldlaufer *et al.*, 2014). For the assays described below, individual adults of both sexes were used, and these had not been fed for 8 days prior to use.

Chemicals

(*E*)-2-Hexenal and (*E*)-2-octenal were obtained from Bedoukian Research, Inc. (Danbury, Connecticut). Their purities were determined by gas chromatography to be 99.7% and 97.6%, respectively. Spectral grade acetone (Honeywell Burdick & Jackson, Morristown, New Jersey) was used for all aldehyde dilutions, and for untreated controls.

Behavioural bioassays

Glass Petri dish (150×20 mm) bottoms were lined with a single sheet of filter paper (Whatman® No. 1; GE Healthcare Life Sciences, U.K.) and used as assay arenas in all experiments.

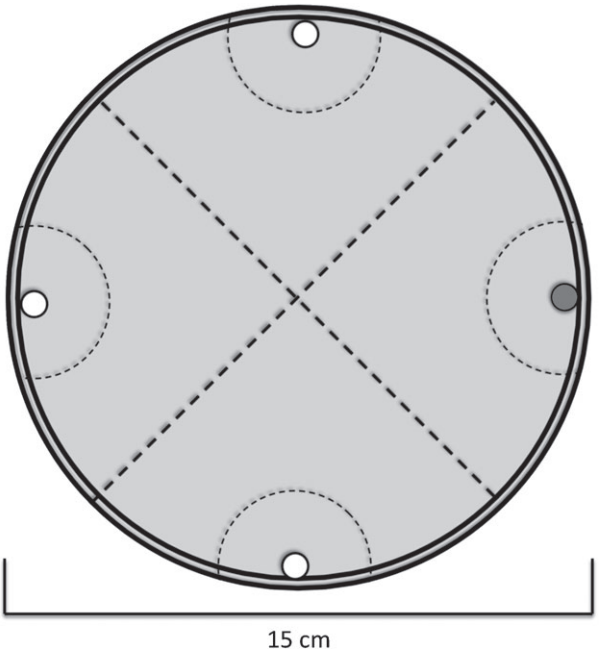

Fig. 1. Petri dish arena (diameter 15 cm) layout and dimensions used for assessing response of bed bugs *Cimex lectularius* to synthetic aldehydes. Four 6-mm disks were placed at the perimeter of arena at the 3, 6, 9 and 12 o'clock positions. One disk (shaded) was impregnated with a dilution of (*E*)-2-hexenal and (*E*)-2-octenal or acetone. Arenas were divided into four equal zones (dashed lines): the treated zone containing the impregnated disk and three untreated zones. Within each zone, a smaller semi-circle subzone (diameter 4 cm) was defined. An individual bed bug was placed in the centre of the arena at the start of each experiment.

Each arena was divided into four equal zones, and further delineated into semi-circular subzones of approximately 40 mm diameter, around each of four disks (Fig. 1). One treated and three untreated 6-mm disks (Whatman, Grade AA) were placed at the perimeter of each arena at the 3, 6, 9 and 12 o'clock positions (Fig. 1). Following a 5-min acclimation period that consisted of the bed bug being contained in the centre of the assay arena under an inverted pipette tip, individual adult *C. lectularius* were released in the arena and their movements were recorded for 1 h. To minimize positional effects, the location of the aldehyde-treated disk was repositioned for each replication. Bed bugs were used only once and, after each trial, the filter paper lining the bottom of the arena was replaced after cleaning the Petri dishes with acetone to reduce the possibility of bed bug-related cues affecting subsequent assays. Assays were conducted under ambient temperature and humidity conditions; the acclimation period and the 1 h assay were conducted in darkness.

Because adults of the common bed bug produce about a 7 : 3 ratio of (*E*)-2-hexenal to (*E*)-2-octenal, and nymphs produce these compounds in approximately the opposite relative amounts, treated disks were impregnated with a mixture of (*E*)-2-hexenal and (*E*)-2-octenal (1 : 1) in acetone to yield 0.004, 0.04, 0.4, 40 or 400 µg of aldehyde blend in each disk. Sixteen

replicates (eight per sex) were conducted for each amount. Acetone was used to treat control disks. Disks were allowed to dry for approximately 15 m before use.

To assess the duration that the aldehyde blend remained attractive, 0.04 μg of the aldehyde blend was used. Treated disks were assayed in the arena at 0, 1, 2, 3 or 24 h post-aldehyde treatment. Twelve replicates (six per sex) were used for each time point. An acetone-treated disk served as a control.

Video analysis

A high-resolution FireWire monochrome camera (Noldus Part No. XVID-002A; Noldus Information Technology Inc., Leesburg, Virginia) with a varifocal lens (Computar model H2Z0414C-MP, 4–8 mm, F 1.4; Computar, Cary, North Carolina) was used to monitor bed bug movement. The camera was suspended 30 cm above the arena, and light for the recordings were provided by an infrared LED illuminator (Axton model AT-8; AxtonTech, North Salt Lake, Utah) attached to the camera.

All video tracking was saved to a computer and analyzed using ETHOVISION® XT automatic tracking software, version 10, Noldus Information Technology Inc.). Bed bugs were tracked at a sampling rate of 15 samples s^{-1}. A threshold movement of 0.25 cm was used as an input filter to eliminate system noise or slight movements not associated with locomotion. A standard calibration of the arena was completed to enable ETHOVISION® to convert the distance between two points from pixels to *x*, *y* coordinates. Data were visualized as movement paths (tracks) or as graphical representations of the subject's position where the frequencies of specific positions are represented as colours ('heat maps'). Heat maps facilitated the identification of clustered data points.

A large number of candidate variables were captured by ETHOVISION®, many of them correlated. The distribution of these variables was also visually examined across the amounts and sometimes appropriate transformations were made (e.g. square root or log), mostly to correct strongly right-skewed variables. The pool of candidate variables was increased further by including the square of some of the variables (quantitative variables that were selected in preliminary analyses), following standardization to mean zero, unit SD, because of the possibility that variables that were optimal for separating amounts did so at intermediate levels. All quantitative variables, following any transformation (or no transformation), were standardized to mean zero, unit SD. A composite score was created using the selected variables, as described below. The variables used as contributors to at least one composite score were: log of minimum distance to treatment disk, log of minimum distance to treatment disk squared, mean distance to treatment disk, mean distance to treatment disk squared, velocity in treatment zone, square root of velocity in treatment zone, total time in treatment zone, the proportion of the number of times the bed bug entered the treated zone (versus all zones), the mean time the bed bug spent in the treated subzone, and total time the bed bug spent in treated subzone. All original units were in centimetres and seconds.

Heat maps from the ETHOVISION® tracking system were also used to 'score' where individual bed bugs spent the majority of their time. Three binary variables were created for the largest cluster of locations on the heat map, which were generally located on the periphery of the Petri dish. A glass template the same size as the entire heat map was divided into quarter (one-eighth on either side of the treated disk) and half (one-quarter on either side of the treated disk) sections, and placed over the treated disk indicator of the heat map. Individual bed bugs were scored as either 'on' the treated disk, 'very close' (within one-quarter of the treated disk) or 'close' (within one-half of the treated disk). These are exclusive categories (not cumulative) (i.e. a bug that was 'on' was not included in the 'very close' and 'close' counts).

Statistical analysis

Data were analyzed as described by Kramer *et al.* (2009), using canonical discriminant analysis to construct a weighting system for concurrently measured behavioural variables. Optimal weightings of behaviours (i.e. those that best differentiate among the treatments) were used to determine composite scores. Bed bugs that behaved similarly produced similar composite scores, whereas treatment groups inducing different behaviours produced different composite scores.

The procedure is outlined below. First, a stepwise procedure [PROC STEPDISC, SAS, version 9.3; SAS Institute Inc., Cary, North Carolina) or the function, greedy.wilks, in the R package (R Core Team, 2014), klaR (Weihs *et al.*, 2005); these gave similar results] was used to identify a subset of variables useful for creating a composite score that separated the stimuli. Quantitative variables were standardized to mean zero and unit SD so that each would contribute on the same scale to a composite scale, making the interpretation of weights (loadings) easier. Next, discriminant analysis using the R function, lda from the MASS package (Venables & Ripley, 2002) was used to create composite scores. It was found that the first two composite scores (linear discriminant axes) captured at least 80% of the explainable variation for separating amounts. In preliminary analyses, it was clear that males and females differed, even with respect to which variables were selected for the composite score, and so the analyses were carried out separately for the two sexes. Analyses of the time delay data (the duration for which the effect of volatiles from the aldehyde mixture at the most attractive amount could be detected) was performed using the same methodology; however, only one dimension (attractancy) of the composite score was found to be useful.

For these composite scores, both the Anderson–Darling test for normality ($P > 0.05$) and the Bartlett test of homogeneity of variances ($P > 0.05$) were met for all treatment groups. Thus, analysis of variance (ANOVA) was used in R (R Core Team, 2014) to determine how the amounts differed in their effects. Mean separations were carried out using the 'multcomp' package in R (Hothorn *et al.*, 2008) using the default single-step method.

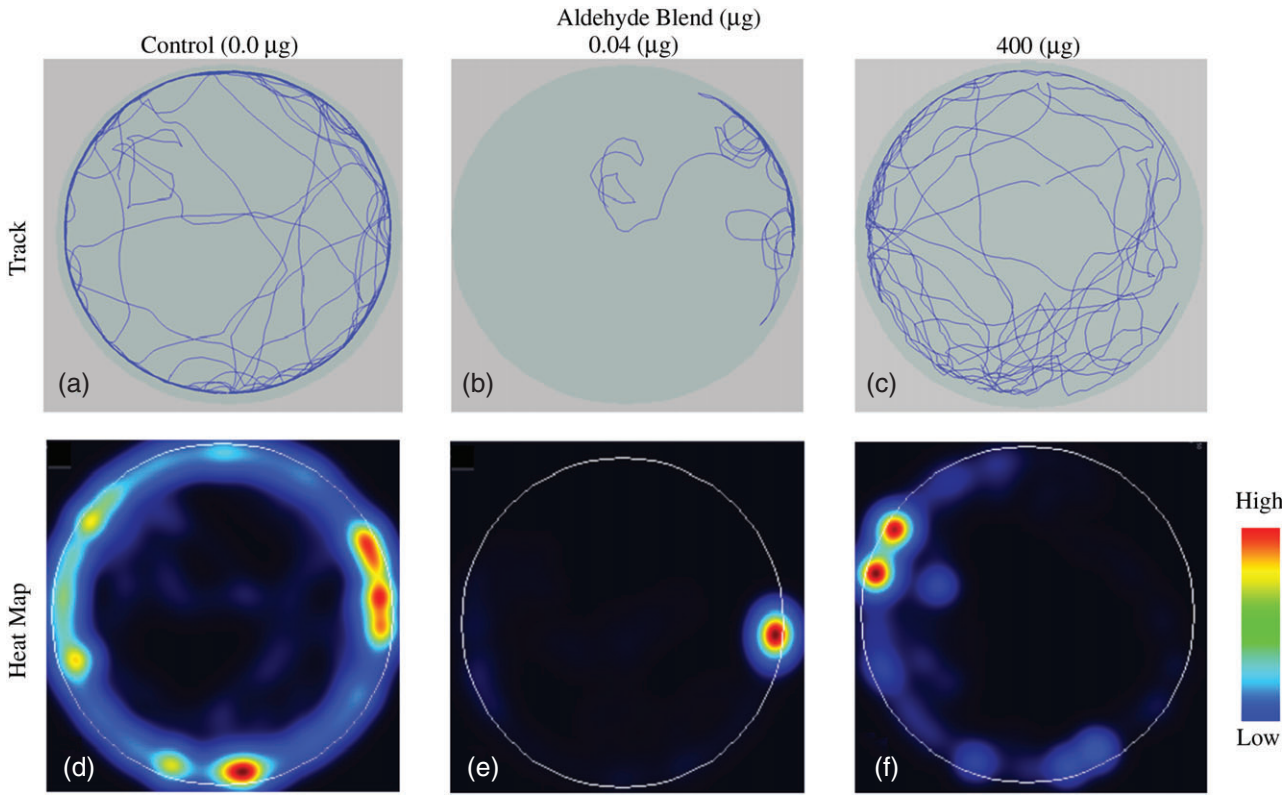

Fig. 2. Representative tracks and 'heat maps' showing movement of individual bed bugs *Cimex lectularius* over a 1-h period in arena (diameter 15 cm). (a–c) Recorded *C. lectularius* tracks for 0, 0.04 and 400 µg aldehyde blend inoculated disks, respectively. (d–f) 'Heat map' visualizations for 0, 0.04 and 400 µg aldehyde blend inoculated disks, respectively. ETHOVISION® generated 'heat maps' visualize a subject's frequency at specific positions based on a colour gradient. Clustered data points appear near the red end of the gradient scale. Treated disks are at the 3 o'clock position in the arena.

Results

Visualization of bed bug tracks showed differences in movement paths and time spent in zones relative to aldehyde-treated disks (Fig. 2). The discriminant analyses produced optimal weights to best differentiate the amounts based on the measured locomotory behaviours of bed bugs in the Petri dish. The weights (loadings) of individual bed bug behaviours for composite score construction using all treatment groups for males and females are shown in Table 1.

Based on composite scores, there was a significant effect of aldehyde amount for both males and females on the first two composite score dimensions (males: $F = 9.55$, d.f. $= 5, 42$, $P < 0.001$. $F = 5.34$, d.f. $= 5, 42$, $P < 0.001$; females: $F = 4.55$, d.f. $= 5, 42$, $P = 0.002$. $F = 4.16$, d.f. $= 5, 42$, $P = 0.004$). Four of the original variables were found to be useful for males to discriminate amounts and three for females (Table 1). For both males and females, the first dimension of the composite score appears to capture repellency or a related variable because high amounts tended to differ from others (Fig. 3), with amounts of 40 µg (females) and 400 µg (males) separating out from the others, and with males showing clearer separation. For males, the two most important variables were minimum distance to treatment and velocity in treatment zone, positively correlated with the first dimension of the composite score, which increased for higher amounts. For females, minimum distance to treatment and mean distance to treatment were important, with the first positively correlated with the first dimension of the composite score, and the second negatively correlated. The second dimension of the composite score appears to capture attractiveness for the amount of 0.04 µg, which again is clearer for males (Fig. 4). For males, the most important contributor was the variable 'on' (positively correlated). Five of the eight males were positive for the variable, 'on', as were two of the eight females. The next largest contributor was mean distance to treatment, squared (negatively correlated), and so intermediate mean distances to treatment were positively associated with this dimension. For females, the mean distance to treatment was negatively correlated, and so shorter mean distances to treatment were positively associated with this dimension.

As noted above, and as shown in Figure 3, the patterns of the composite scores for males and females were similar despite being based on different variables. If a superset of all variables common to both sexes were used to create the composite scores, the same overall pattern emerged, with weightings differing between the sexes (results not shown).

For duration (time delay) assays, where bed bugs were exposed to a treated disk (0.04 µg) at different times

Table 1. Weights for *Cimex lectularius* behaviours used in constructing composite scores for the aldehyde amount bioassay and duration bioassay.

	Weights[a]	
Locomotory behavior	Dimension 1 (repellent)	Dimension 2 (attractant)
Amount bioassay		
Males		
Log (minimum distance to treatment)	1.04	0.72
'On' treatment disk	0.01	1.18
Mean distance to treatment[b]	0.05	−0.81
Square root (velocity in treatment zone)	0.77	−0.30
Females		
Mean distance to treatment	−0.68	−1.26
Log (minimum distance to treatment)[b]	0.30	−0.15
Log (minimum distance to treatment)	1.06	−0.01
Duration bioassay		
Males		
Total time in treatment zone[b]		−1.49
Proportion of the frequency entering treated zone		0.76
Velocity in treatment zone		0.09
Females		
Total time in treatment zone		−1.60
Mean time in treated subzone		2.96
Total time in treated subzone		−2.19

[a]Weights were determined using the canonical discriminant functions (R MASS lda function) and used to create the composite scores. For the 'Amount Bioassay', dimension 1 is interpreted as a 'repellent' axis; dimension 2 is interpreted as an 'attractant' axis. For the 'Duration Bioassay', only one dimension was useful, and this is interpreted as an 'attractant' axis. Behaviours with larger absolute values better separate the responses. All behaviours were standardized to mean zero, unit SD prior to analyses.

[b]Zones and subzones are defined in the text.

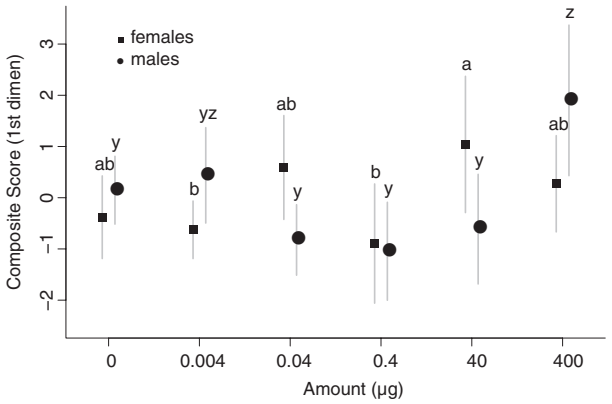

Fig. 3. Plot of 'repellency' (first dimension) of composite score of the amount of aldehyde blend [(*E*)-2-hexenal:(*E*)-2-octenal, 1 : 1, in micrograms per treated disk] to *Cimex lectularius*. The mean scores for both males and females are shown. Vertical bars indicate 1 SD. Amounts with the highest scores were most repellent to bed bugs. Amounts with the same lowercase letters do not differ significantly.

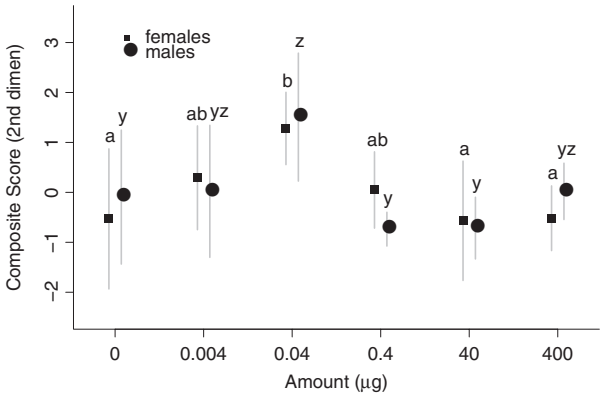

Fig. 4. Plot of 'attractancy' (second dimension) of composite score of the amount of aldehyde blend [(*E*)-2-hexenal:(*E*)-2-octenal, 1 : 1, in micrograms per treated disk] to *Cimex lectularius*. The mean scores for both males and females are shown. Vertical bars indicate 1 SD. Amounts with the highest scores were most attractive to bed bugs. Amounts with the same lowercase letters do not differ significantly.

post-treatment, three variables were used to construct the discriminant function for each sex, and these differed from each other and from those used in amount bioassay (Table 1). Total time in treatment zone was common to both sexes and with similar negative weights. The variables proportion of the frequency of the bug entering the treated zone and velocity in treatment zone were selected for males (the latter with little contribution), and two area variables, the average time and total time spent in the treated subzone, were selected for females with opposite signs. If not for three observations, the average and total time spent in the treated subzone were relatively uncorrelated, and the sum of these two variables, after multiplying by their loadings, produced a pattern similar to the results from all three variables (not shown). The pattern was still visible, although less clear, if only the total time spent in the treated subzone or treatment zone was used. An ANOVA for this first dimension

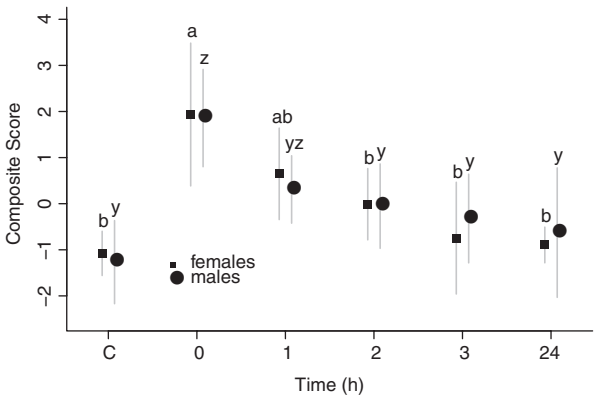

Fig. 5. Plots of composite score ('attractancy') for 0.04 µg of aldehyde blend [(*E*)-2-hexenal:(*E*)-2-octenal, 1 : 1] to *Cimex lectularius*. Time axis indicates the delay in hours prior to conducting bioassays. The mean scores for both males and females are shown. Vertical bars indicate 1 SD. Times with the highest scores were most attractive to bed bugs. Times with the same lowercase letters do not differ significantly. Control (C) consisted of acetone-treated disks.

of the composite score was significant for both sexes (males: $F = 6.70$, d.f. = 5, 29, $P < 0.001$; females: $F = 7.86$, d.f. = 5, 29, $P < 0.001$). Despite the different variables used to construct the composite scores, the patterns of males and females were remarkably similar (Fig. 5). For both sexes, only the 0-h delay differed significantly ($P < 0.05$) from the acetone control, and the 0- and 1-h delays did not differ significantly from each other (Fig. 5). Although time was considered a qualitative variable (i.e. a smooth function was not fit to the time variable), the results show a clear drop-off in score after the 0-h delay as an increasing function of time.

Discussion

The bed bugs *C. lectularius* in the present study are attracted to 0.04 µg of a mixture (1 : 1) of the two major common bed bug defensive secretions, (*E*)-2-hexenal and (*E*)-2-octenal. This response is consistent with aggregation cues produced by low amounts of bed bug volatiles (Olson *et al.*, 2009; Weeks *et al.*, 2013). Siljander *et al.* (2008) report that a mixture of eight additional compounds in addition to (*E*)-2-hexenal and (*E*)-2-octenal is essential for *C. lectularius* aggregation, although a more recent study (Gries *et al.*, 2014) reports that five compounds are necessary for aggregation in the common bed bug. However, both studies include (*E*)-2-hexenal and (*E*)-2-octenal, and the present study finds that a mixture of both of these compounds is sufficient to induce attraction. The differential response of males is much clearer than that of females; this tendency for females to be less responsive to volatiles is observed in other studies using the common bed bug (Domingue *et al.*, 2010; Weeks *et al.*, 2013). The female-specific behaviour may be associated with fitness costs. Although aggregation offer several advantages to an individual (Benoit *et al.*, 2007; Saenz *et al.*, 2014), strong sexual conflict (Morrow & Arnqvist, 2003) may drive females away from high density aggregations (Pfiester *et al.*, 2009). Interestingly, the variables selected to separate the amounts differ between the sexes. Mean distance to treatment is the only important variable correlated with this dimension for females, whereas others are also important for males. This suggests that females respond to the attractive amount in a simpler way, spending more time near but not necessarily on the source, whereas the response of males is more complicated because several tend to stay as close as possible to the source (the 'on' variable). Based on these results, males might be expected to outnumber females when approaching the centre of a bed bug aggregation. This may also influence optimal trap design, which may need components that accommodate the differences with respect to how males and females respond to a source of the attractive amount.

Under laboratory conditions, high doses of (*E*)-2-hexenal and (*E*)-2-octenal cause dispersal of *C. lectularius* (Levinson & Bar Ilan, 1971). Emissions of volatiles of the closely-related tropical bed bug *C. hemipterus* cause a similar dispersal response (Liedtke *et al.*, 2011). The behavioural variables in the present study capture this in the first dimension of the composite score, although optimal repellent doses and selected behaviours also differ by sex. Saturation of pheromone receptors may differ for the two sexes, and this could account for some of observed differences (Benoit *et al.*, 2009). In addition to distance measurements, the movement patterns of males change; increased velocity in the treatment zone is positively correlated with this dimension of their composite score. Females do not approach this high amount treatment area (both the log of minimum distance and its square are positively correlated with this dimension).

Pheromones could be used for (i) monitoring, (ii) mass trapping or (iii) be incorporated with insecticides to attract and kill bed bugs (Benoit *et al.*, 2009). The results of the present study show that the limit of effectiveness of an aldehyde-treated disk is 1 h, and it is hoped that slow release formulations can be developed. This is important because the feasibility of practical field use is highlighted. It should also be noted that the trials in the present study are conducted in a controlled environment; human or household odours might impact attraction behaviours. In addition, there is little air movement in the Petri dish, and so the gradient of the volatiles in the Petri dish is likely much weaker than it would be if the aldehyde source were to be exposed to room air currents. Even so, the bed bugs are attracted to the source. It is expected that, in a room with a steeper gradient of volatiles, the behaviours observed would be even more evident (i.e. there would be more attraction to the source of the volatiles).

Studying the chemical ecology of bed bugs provides insight into relevant signalling cues, with direct implications for bed bug management practices. The present study highlights specific behavioural variables that can be used to determine adult bed bug responses to synthetic aldehydes. An understanding of attraction and repellent behaviour is necessary for developing an effective monitor that can be used as part of a successful management strategy, although release formulations will be a critical part of the development of any monitor.

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References

Aak, A., Rukke, B.A., Soleng, A. & Rosnes, M.K. (2014) Questing activity in bed bug populations: male and female responses to host signals. *Physiological Entomology*, **39**, 199–207.

Benoit, J.B., Del Grosso, N.A., Yoder, J.A. & Denlinger, D.L. (2007) Resistance to dehydration between bouts of blood feeding in the bed bug, *Cimex lectularius*, is enhanced by water conservation, aggregation, and quiescence. *American Journal of Tropical Medicine and Hygiene*, **76**, 987–993.

Benoit, J.B., Phillips, S.A., Croxall, T.J. *et al.* (2009) Addition of alarm pheromone components improves the effectiveness of desiccant dusts against *Cimex lectularius*. *Journal of Medical Entomology*, **46**, 572–579.

Centers for Disease Control and Prevention (2011) Acute illnesses associated with insecticides used to control bed bugs – seven states, 2003–2010. *Morbidity and Mortality Weekly Report*, **60**, 1269–1274.

Collins, R. (1968) Carbonyl compounds produced by the bed bug, *Cimex lectularius*. *Annals of the Entomological Society of America*, **61**, 1338–1340.

Cooper, R., Wang, C. & Singh, N. (2014) Accuracy of trained canines for detecting bed bugs (Hemiptera: Cimicidae). *Journal of Economic Entomology*, **107**, 2171–2181.

Domingue, M.J., Kramer, M. & Feldlaufer, M.F. (2010) Sexual dimorphism of arrestment and gregariousness in the bed bug (*Cimex lectularius*) in response to cuticular extracts from nymphal exuviae. *Physiological Entomology*, **35**, 203–213.

Feldlaufer, M.F., Domingue, M.J., Chauhan, K.R. & Aldrich, J.R. (2010) 4-Oxo-Aldehydes from the dorsal abdominal glands of the bed bug (Hemiptera: Cimicidae). *Journal of Medical Entomology*, **47**, 140–143.

Feldlaufer, M.F., Harlan, H.J. & Miller, D.M. (2014) Laboratory rearing of bed bugs. *Rearing Animal & Plant Pathogen Vectors* (ed. by K. Mamamorosch and F. Mahmood), pp. 118–140. CRC Press, Boca Raton, Forida.

Gries, R., Britton, R., Holmes, M. *et al.* (2014) Bed bug aggregation pheromone finally identified. *Angewandte Chemie International Edition*, **53**, 1–5.

Harraca, V., Ryne, C. & Ignell, R. (2010) Nymphs of the common bed bug (*Cimex lectularius*) produce anti-aphrodisiac defense against conspecific males. *BMC Biology*, **8**, 121.

Hothorn, T., Bretz, F. & Westfall, P. (2008) Simultaneous inference in general parametric models. *Biometrical Journal*, **50**, 346–363.

Jones, S.C. & Bryant, J.L. (2012) Ineffectiveness of over-the-counter total-release foggers against the bed bug (Heteroptera: Cimicidae). *Journal of Economic Entomology*, **105**, 957–963.

Kramer, M., Weldon, P.J. & Carroll, J.F. (2009) Composite scores for concurrent behaviours constructed using canonical discriminant analysis. *Animal Behaviour*, **77**, 763–768.

Levinson, H.Z. & Bar Ilan, A.R. (1971) Assembling and alerting scents produced by the bedbug *Cimex lectularius* L. *Experientia*, **27**, 102–103.

Levinson, H.Z., Levinson, A.R. & Maschwitz, U. (1974) Action and composition of the alarm pheromone of the bedbug *Cimex lectularius* L. *Die Naturwissenschaften*, **61**, 684–685.

Liedtke, H.C., Åbjörnsson, K., Harraca, V. *et al.* (2011) Alarm pheromones and chemical communication in nymphs of the tropical bed bug *Cimex hemipterus* (Hemiptera: Cimicidae). *PLoS ONE*, **6**, e18156.

Marx, R. (1955) Über die Wirtsfindung und die Bedeutung des artspezifischen Duftstoffes bei *Cimex lectularius* Linné. *Zeitschrift für Parasitenkunde*, **17**, 41–73 (in German)..

Mendki, M.J., Ganesan, K., Parashar, B.D. *et al.* (2014) Aggregation responses of *Cimex hemipterus* F. to semiochemicals identified from their excreta. *Journal of Vector Borne Diseases*, **51**, 224–229.

Morrow, E.H. & Arnqvist, G. (2003) Costly traumatic insemination and a female counter-adaptation in bed bugs. *Proceedings of the Royal Society of London Series B: Biological Sciences*, **270**, 2377–2381.

Olson, J.F., Moon, R.D. & Kells, S.A. (2009) Off-host aggregation behavior and sensory basis of arrestment by *Cimex lectularius* (Heteroptera: Cimicidae). *Journal of Insect Physiology*, **55**, 580–587.

Pfiester, M., Koehler, P.G. & Pereira, R.M. (2008) Ability of bed bug-detecting canines to locate live bed bugs and viable bed bug eggs. *Journal of Economic Entomology*, **101**, 1389–1396.

Pfiester, M., Koehler, P.G. & Pereira, R.M. (2009) Effect of population structure and size on aggregation behavior of *Cimex lectularius* (Hemiptera: Cimicidae). *Journal of Medical Entomology*, **46**, 1015–1020.

R Core Team (2014) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Austria [WWW document]. URL http://www.R-project.org/ [accessed on 1 August 2015].

Ryne, C. (2009) Homosexual interactions in bed bugs: alarm pheromones as male recognition signals. *Animal Behaviour*, **78**, 1471–1475.

Saenz, V.L., Santangelo, R.G., Vargo, E.L. & Schal, C. (2014) Group living accelerates bed bug (Hemiptera: Cimicidae) development. *Journal of Medical Entomology*, **51**, 293–295.

Schildknecht, H., Holoubek, K., Weis, K.H. & Krämer, H. (1964) Defensive substances of the arthropods, their isolation and identification. *Angewandte Chemie International Edition*, **3**, 73–82.

Siljander, E., Penman, D., Harlan, H. & Gries, G. (2007) Evidence for male- and juvenile-specific contact pheromones of the common bed bug *Cimex lectularius*. *Entomologia Experimentalis et Applicata*, **125**, 215–219.

Siljander, E., Gries, R., Khaskin, G. & Gries, G. (2008) Identification of the airborne aggregation pheromone of the common bed bug, *Cimex lectularius*. *Journal of Chemical Ecology*, **34**, 708–718.

Singh, N., Wang, C., Cooper, R. & Liu, C. (2012) Interactions among carbon dioxide, heat, and chemical lures in attracting the bed bug, *Cimex lectularius* L. (Hemiptera: Cimicidae). *Psyche*, **2012**, 1–9.

Vaidyanathan, R. & Feldlaufer, M.F. (2013) Bed bug detection: current technologies and future directions. *American Journal of Tropical Medicine and Hygiene*, **88**, 619–625.

Venables, W.N. & Ripley, B.D. (2002) *Modern Applied Statistics with S*, 4th edn. Springer, New York, New York.

Wang, C., Saltzman, K., Chin, E. *et al.* (2010) Characteristics of *Cimex lectularius* (Hemiptera: Cimicidae), infestations and dispersal in a high-rise apartment building. *Journal of Economic Entomology*, **103**, 172–177.

Wang, C., Tsai, W.-T., Cooper, R. & White, J. (2011) Effectiveness of bed bug monitors for detecting and trapping bed bugs in apartments. *Journal of Economic Entomology*, **104**, 274–278.

Weeks, E.N.I., Birkett, M.A., Cameron, M.M. *et al.* (2011a) Semiochemicals of the common bed bug, *Cimex lectularius* L. (Hemiptera: Cimicidae), and their potential for use in monitoring and control. *Pest Management Science*, **67**, 10–20.

Weeks, E.N.I., Logan, J.G., Gezan, S.A. *et al.* (2011b) A bioassay for studying behavioural responses of the common bed bug, *Cimex lectularius* (Hemiptera: Cimicidae) to bed bug-derived volatiles. *Bulletin of Entomological Research*, **101**, 1–8.

Weeks, E.N.I., Logan, J.G., Birkett, M.A. *et al.* (2013) Tracking bed bugs (*Cimex lectularius*): a study of the effect of physiological and extrinsic factors on the response to bed bug-derived volatiles. *Journal of Experimental Biology*, **216**, 460–469.

Weihs, C., Ligges, U., Luebke, K. & Raabe, N. (2005) klaR analyzing German business cycles. *Data Analysis and Decision Support* (ed. by D. Baier, R. Decker and L. Schmidt-Thieme), pp. 335–343. Springer-Verlag, Germany.

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