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NESTING AND QUESTING ACTIVITY IN BED BUG POPULATIONS: MALE AND FEMALE RESPONSES TO HOST SIGNALS

[1]ANDERS AAK, [1]BJØRN ARNE RUKKE, [1]ARNULF SOLENG, AND [2]MARTE K. ROSNES

[1]Norwegian Institute of Public Health – Department of Pest Control. Lovisenberggata 8, Postboks 4404 Nydalen, NO-0456 Oslo, Norway
[2]County Governor Sogn og Fjordane, Njøsavegen 2, 6863 Leikanger, Norway

Abstract A large-arena bioassay is used to examine gender differences in the spatiotemporal patterns of bed bug, *Cimex lectularius* Latreille 1802, behavioural responses to either a human host or CO_2. After release in the center of the arena, 90% of the newly fed bed bugs move to hiding places in the corners within 24 h. They require 3 days to settle down completely in the arena, with generally low activity levels and the absence of responses to human stimuli for 5 days. After 8-9 days, persistent responses can be recorded. Gender differences are observed, in which females are more active during establishment, respond faster after feeding, expose themselves more than males during the daytime, and respond more strongly to the host signal. The number of bed bugs that rest in harbourages is found to vary significantly according to the light setting and gender. Both genders stay more inside the harbourages in daylight compared with the night, and males hide more than females during the daytime but not during the night. The spatial distribution of the bed bugs is also found to change with the presence of CO_2, and peak aggregation around the odour source is observed after 24 min. Both male and female bed bugs move from the hiding places or the border of the arena toward the centre where the CO_2 is released. Peak responses are always highest during the night. Bed bug behaviour and behaviour-regulating features are discussed according to control methods.
Key words Bed bug, *Cimex lectularius*, *Cimex hemipterus*, host location.

INTRODUCTION

Bed bugs (*Cimex lectularius*) are closely connected to their host. Each nymphal stage must have a blood meal to proceed in its lifecycle, and adults need blood to successfully produce offspring (Reinhardt and Siva-Jothy, 2007; Usinger, 1966). During feeding, the bed bugs elevate their mortality risk by exposing themselves to the host when leaving their concealed and safe harbourages. The time spent on or around the host is therefore minimized, and the bed bugs remain hidden from humans for the majority of their lives. This cryptic way of living is one of the major challenges in bed bug control because it reduces our chances of bed bug detection and makes the efficient application of killing agents difficult. Bed bugs are currently increasing worldwide (Davies et al., 2012). To overcome pesticide resistance (Kilpinen et al., 2011; Romero et al., 2007; Tawatsin et al., 2011; Zhu et al., 2010) and cope with their concealed biology, improved control strategies are needed (Koganemaru and Miller, 2013; Weeks et al., 2010). To find potential bed bug weaknesses that can be exploited to develop efficient integrated pest management solutions, increased knowledge about bed bug behaviour is necessary.

Bed bug behaviour consists of sub-social elements, such as aggregation and alarm signals (Levinson et al., 1974; Olson et al., 2009; Siljander et al., 2008). They also benefit from nesting activity, in which high-density accumulations reduce water loss (Benoit et al., 2007). Odours from blood secretion, feces, and exuviae combine with residues from the alarm signals to produce a volatile cue that in conjunction with tactile stimuli allows bed bugs to aggregate in their nests after feeding on their host (Domingue et al., 2010; Olson et al., 2009; Siljander et al., 2008). Host locations also depend on olfactory cues (Anderson et al., 2009; Harraca et al., 2012; Singh et al., 2012), and the bed bug appears to be governed by an assembly of volatiles that regulate movement and resting.

Awareness of the complexity of olfactory signals (Bruce et al., 2005; Lazzari, 2009; Logan and Birkett, 2007) and increased knowledge of olfactory-mediated bed bug behaviour (Siljander, 2006; Weeks et al., 2010) may be utilized as tools to improve the efficiency of control methods (Benoit et al., 2009). In many other pest situations, semiochemicals are used for early detection, push-pull strategies, mass trapping, luring and killing strategies, luring and infecting strategies, and mating disruption (Agelopoulos et al., 1999; Cook et al., 2007; El-Sayed et al., 2009; El-Sayed et al., 2006; Witzgall et al., 2010). The spatiotemporal patterns of bed bug questing, together with descriptions of life stages and gender-specific responses to different semiochemicals need to be considered to properly time and direct treatment. Some disparities in bed bug activity and movement are known across sexes and between life stages (Domingue et al., 2010; Pfiester et al., 2009; Romero et al., 2010; Weeks et al., 2011), but gender differences in host perception have not been investigated, although this is considered an important applied aspect of semiochemical control (El-Sayed et al., 2006).

Laboratory behavioural studies are integral parts of the development of semiochemical control methods, and bioassays should allow the insects to act in a nearly natural way. This is difficult to achieve with bed bugs, however, because removal from stock cultures, handling, and transfer to the bioassay clearly interfere with the concealed biology of the insect and may consequently influence their responses. Behavioural studies that described bed bug activity have often been performed in Petri dishes or in small-scale experimental arenas where behaviour is measured during a limited time span (Harraca et al., 2012; Olson et al., 2009; Pfiester et al., 2009; Romero et al., 2010; Weeks et al., 2013; Weeks et al., 2011). These experimental setups are valuable tools for identifying basic behavioural elements, but a low spatiotemporal scale may make field application of the results more difficult. Larger-scale arena trials, which more closely mimic a natural indoor bed bug pest situation, provide information about the attraction potential of CO_2 and chemical lures (Anderson et al., 2009; Singh et al., 2013; Singh et al., 2012; Wang et al., 2009; Wang et al., 2013).

To fully describe natural bed bug responses, all regulating factors need to be considered, and a bioassay should allow bed bugs to behave as naturally as possible. In the present study, we seek to describe a large-scale arena bioassay to identify elements that are necessary to produce quantifiable behavioural responses to host signals. Gender and day-night differences in responses are described, and we investigate the effect of the time since feeding on the level of responsiveness. Using the arena bioassay, we provide detailed measures of the bed bugs' response profiles when stimulated by volatiles from a human. We also investigate responses to a pure CO_2 point source to describe its effects on spatial distribution in the arena.

MATERIALS AND METHODS

Insects. The bed bug stock culture was collected in 2009. The initial population consisted of 40 adult specimens that originated from a hotel in Oslo, Norway. Cultures were maintained in 140 ml

polyethylene boxes (7 cm height, 5 cm inner diameter) that contained folded paper towels to provide harbourages and allow the bed bugs to move, mate, and lay eggs. All of the cultures were maintained in a 15 h/9 h light/dark cycle at room temperature (20-22ºC) and 50-60% relative humidity. Stock cultures were fed artificially and on rodents until experiments were performed.

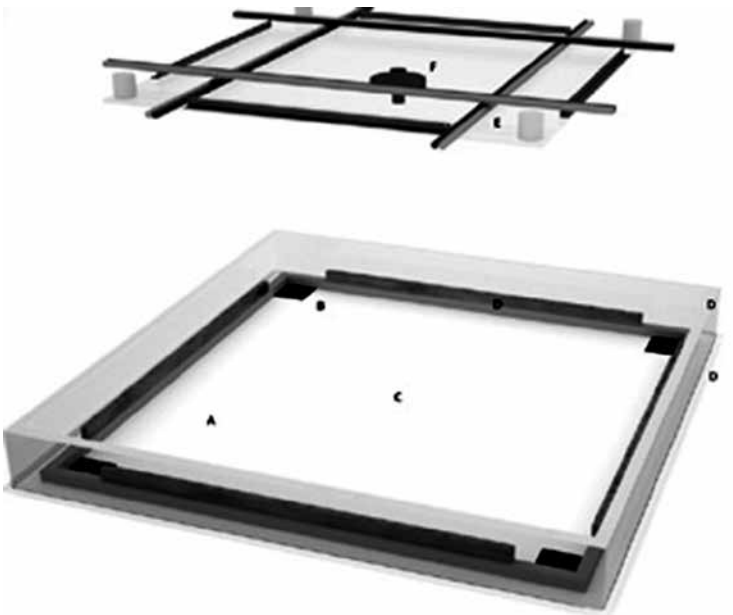

Figure 1. Three-dimensional rendering of the bed bug arena. (A) Area of movement. (B) Harborages. (C) CO2 release point. (D) Safety barriers (polished plastic wall with copper weights, Plexiglas wall, insect glue-coated overhang, and mineral oil-filled duct). (E) White, red, and infrared light sources. (F) Vivotech IP camera.

Bed bug arena. The experimental arena (Figure 1) was placed in a 15 m^2 room (3 m x 5 m) without windows. The room was air-conditioned and maintained at an average (± SE) temperature of 22.3 ± 0.1ºC and relative humidity of 31.7 ± 3.3%. The room was only furnished with a chair and desk, and the walls and ceiling were white or grey to minimize visual cues. All of the light sources were positioned directly above the arena and consisted of a set of eight Plexiglas tubes (1.2 cm diameter, 110 cm length), each with 96 light-emitting diode (LED) lights positioned 1 cm apart (8 W, Northlight LED, Clas Ohlson, Oslo, Norway) and four infrared (IR) lamps (Ecoline IR illuminator, TV6700, ELFA, Kolbotn, Norway). The eight tubes were placed in a grid fashion and the 768 LED lights were directed toward the arena. The four IR lamps were directed toward the ceiling. This lighting setup provided shadow-free and even light conditions in the arena to facilitate night and day observations of the bed bugs. Half of the Plexiglas tubes were coated with red plastic foil. Day was simulated by turning all of the light sources on. Night was simulated by turning on only the red tubes and IR lights. The light cycle was 15 h/9 h (light/dark) to keep the conditions similar to the rearing facilities and what is commonly found in a bedroom. The dark cycle began at 15:00 hours and lasted until 00:00 hours. A day- and night-vision Internet Protocol (IP) camera (Vivotek, FD 8361, Multicom, Åmli, Norway) was used to record a 1200 x 1600 pixel video with five frames per second. Sharp night videos were obtained using the built-in IR-cut filter and iris technology. The recording software was Vivotek web-client, version 1.00.

The bed bug arena was placed 90 cm above the floor. The arena base was constructed from a 2-cm-thick white plastic plate (Polyoksymetylen, copolymer: 30 kg/m^2, Plastkompaniet A/S, Oslo, Norway) that measured 150 cm x 150 cm. A 15 cm high and 2 mm thick Plexiglas wall, with a 2 cm insect glue-coated overhang, surrounded the arena. As an additional safety measure to prevent bed bug escape, the wall was bordered by a duct filled with mineral oil. An inner frame constructed from polished

hard plastic defined the borders of the area where the bed bugs could move (130 cm x 130 cm). This frame was held in place by brass rods that kept in place one white piece of paper (134-white seamless background paper, 175 g/m^2) that covered the arena floor and provided grip for the moving bed bugs. In six experimental series, the arena was used in its entirety. In two experimental series, the inner part of the arena was split in two halves (130 cm x 65 cm) by an additional plastic wall. Harbourages, made from 10 cm x 10 cm transparent dark-red Plexiglas (Plexiglas, GS268, Plastkompaniet A/S, Oslo, Norway), were positioned in each of the corners of the arena. The Plexiglas harbourage had a corner that faced the centre of the arena, elevated approximately 3 mm.

Experimental protocol. Fifth instar nymphs were fed on rodents until fully engorged; which provided newly emerged adults after 2 weeks. The emerged adults were sexed by registration of the genitalia and offered a rodent blood meal. Immediately after feeding, an equal number of fully engorged male and female bed bugs were transferred to the experimental room and released in the arena. The room was left empty on the initial day to allow the bed bugs to establish themselves without interference. During the experimental series, human stimulation was performed by one person who entered the room and sat in a chair next to the bed bug arena or by CO_2 that was presented to the bed bugs by placing a small Petri dish (55 mm diameter) that contained an average (± SE) of 17.1 ± 0.2 g dry ice in the middle of the arena. Each stimulation lasted 30 min. As a control for human stimulation, we used the room with no humans in it. As a control for CO_2 stimulation, we used an empty Petri dish. Experimental bed bugs were only used in one 14-day series and were killed by freezing upon completion of the experiment.

To describe the response of the bed bugs to their natural human host, we initially used five males and five females in the arena. From the second day of experimentation, a human entered the room every third hour from 07:00 hours to 22:00 hours to provide six stimulations per day (Table 1). Three stimulations occurred during the day, and three at night. This experiment lasted 14 days and repeated three times. As a control, three additional 14-day experimental series were performed with no stimulation in the room. To investigate gender differences, the bed bug arena was split into two halves. Ten males were released in one half, and ten females in the other half. From the second day of experimentation until day 4, we did not expect host-initiated activity, and the bed bugs were stimulated only once by a human in the daylight (Table 1). From day 5-9 in the experimental series, a human entered the room every third hour from 10:00 hours to 19:00 hours to provide four stimulations per day, three hours apart. Two stimulations occurred during the day, and two at night. From day 10 to day 14, the four daily human stimulations were replaced by either CO_2 or an empty Petri dish (Table 1). The daily order of CO_2 stimulation and the control were alternated to achieve an overall balance between which treatment being introduced first during both the day and night. The split population experiment was repeated twice.

Video analysis and quantification of behaviour. In all of the experiments, the bed bugs and their activity in the arena were video-recorded. When analysing the video files, we scored behaviour for 1 min every 6 min. This gave a total of 10 min of recording every hour and a total of 5 min of recording during stimulation. During each minute of recording, the total number of bed bugs that occupied the open spaces of the arena, number of bed bugs that rested in their harbourages, and cumulative number of bed bug individuals that moved during the 1 min period of observation were counted. In the split series, the spatial distribution of the bed bugs was investigated when the CO_2 or empty control Petri dish was presented as a point source. Changes in the bed bugs' positions were analysed by dividing the entire arena into an 8 x 8 square grid. The number of individuals in each of the 64 squares (16 cm x 16 cm) was counted in the freeze frame at the beginning of each minute of recording (6 min before stimulation, every sixth minute during stimulation, and 18 min after stimulation).

Table 1. Experimental set-up of the day/night regimen, with methods and periods of stimulation in the bed bug arena in mixed and split populations. White represents day (white, red, and infrared lights on), and grey represents night (only red and infrared lights on). ○ = no stimuli, ●= human, ◎ = empty Petri dish, x = Petri dish with dried ice to release CO_2.

	MIXED POPULATION		**SPLIT POPULATION**		
Stimuli	*Human* (Day 2-14)	*Control* (Day 2-14)	*Human* (Day 2-4)	*Human* (Day 5-9)	*CO_2/Empty Petri dish** (Day 10-14)
00:00-07:00	○	○	○	○	○
07:00-07:30	●	○	○	○	○
07:30-08:00	○	○	○	○	○
08:00-10:00	○	○	○	○	○
10:00-10:30	●	○	●	●	◎
10:30-11:00	○	○	○	○	○
11:00-13:00	○	○	○	○	○
13:00-13:30	●	○	○	●	◎
13:30-14:00	○	○	○	○	○
14:00-16:00	○	○	○	○	○
16:00-16:30	●	○	○	●	◎
16:30-17:00	○	○	○	○	○
17:00-19:00	○	○	○	○	○
19:00-19:30	●	○	○	●	◎
19:30-20:00	○	○	○	○	○
20:00-22:00	○	○	○	○	○
22:00-22:30	●	○	○	○	○
22:30-23:00	○	○	○	○	○
23:00-24:00	○	○	○	○	○
Repetitions:	*14 days n = 3*	*14 days n = 3*	*3 days n = 2*	*5 days n = 2*	*5 days n = 2*

*The order of CO_2/empty Petri dish was alternated daily.

Statistical analysis. Data were analysed using SigmaPlot 12 (Systat Software, San Jose, CA, USA). Data were checked for normality, and multiple comparisons were performed using analysis of variance (ANOVA). Pairwise comparisons were performed using *t*-tests or paired *t*-tests. The level of significance was set to 0.05, and differences between multiple comparisons were identified using the Tukey test. If the tests of normality failed, then we used the nonparametric Mann-Whitney test, Mann-Whitney Rank Sum Test, or Kruskal-Wallis ANOVA.

RESULTS

General activity. No response to human stimulation was observed during the initial period of observation, which was considered establishment in the arena and excluded from further analysis. We observed generally higher activity among females compared with males, and the split populations were two- to three-times more active as the mixed populations during the initial 3 days. Almost all of the bed bugs (90.0 ± 4.9%) moved from the open spaces of the arena where they were released to the corners within 24 h.

Overall activity in the control series with no stimulation was low, with an average (± SE) of 0.02 ± 0.01 individuals that moved during the 10 min observation period per hour in daylight, and 0.62 ± 0.05 individuals moved at night. The bed bugs were active according to the light regimen, and distinct activity peaks could be observed during the night (Figure 2A) when the maximum number of individuals that moved within a 1 min observation period reached five of 10. Overall activity during the human stimulation series was significantly higher than in the control series, in which 0.82 ± 0.07 individuals moved in daylight (Mann-Whitney Rank Sum Test: $T = 85075$, $P < 0.001$) and 1.43 ± 0.13 individuals moved at night (Mann-Whitney Rank Sum Test: $T = 74870$, $P < 0.001$). In the stimulation series, spikes of activity related to human presence could be observed during both the night and day (Figure 2B). Maximum activity was reflected by movement of all 10 individuals in a 1 min period of observation. This occurred at night, either during human stimulation or following the presence of a host.

Responses to host. A change in activity occurred in the mixed populations on day/night 9 in response to human presence (Figure 3A, B). At night, no difference was found between the control and human presence from day 4-8 (paired *t*-test: $t = 0.78$, $P = 0.480$), activity was significantly higher with a human present from day 9 onward (paired *t*-test: $t = 12.84$, $P < 0.001$; Figure 3A). During daylight, activity in the control series was close to zero, and human presence significantly increased activity during both periods (paired *t*-test: day 4-8, $t = 3.53$, $P = 0.012$; day 9-13, $t = 6.69$, $P = 0.003$; Figure 3B).

When males and females were separated, the level of female activity during human stimulation gradually increased until day 8, whereas male activity was low. Females always moved more than males but only significantly more on day 8 (Mann-Whitney Rank Sum Test: $T = 773.5$, $P < 0.001$; Figure 3C) and day 9 (Mann-Whitney Rank Sum Test: $T = 740.5$, $P = 0.002$; Fig. 3C). When humans were replaced with CO_2, this difference in responsiveness persisted, but males increased activity throughout the last 10 days to a similar level as females. A significant gender difference in response to CO_2 was found only on day 10 ($P < 0.020$; Figure 3C).

When the stimulation events were divided into 6 min intervals, we observed a distinct response that began when the host entered the room and when dry ice was placed in the arena. Activity continuously increased until the stimulus was removed and then gradually decreased during the next hour. Peak responses were always the highest during the night. In the mixed population, the response profile appeared to be similar, regardless of night or day, but was less prominent from day 4 to day 8 compared with the interval from day 9 to day 13 (Figure 4A, B). In the split series, only one of five tests exhibited a significant gender difference in response to CO_2, and the data were pooled across sexes to create a response profile (Figure 4C). CO_2 stimulation appeared to be similar to a human odor source with regard to the temporal change in activity, but CO_2 stimulation produced more responders. Night stimulation in the split series occurred during the first half of the night, and a general increase in activity was observed in both the control and stimulation conditions during this period (Figure 4C).

Spatial distribution. Six minutes before stimulation, the number of bed bugs that rested in the four squares that contained a harbourage varied significantly according to the light setting and gender (Kruskal-Wallis ANOVA on Ranks: $H = 36.092$, $df = 3$, $P < 0.001$). The Tukey *post hoc* test revealed that both genders

positioned themselves more in the harbourages in the daylight compared with the night, and males hid more than females during the daytime but not during the night (Figure 5). The spatial distribution of the bed bugs also changed with the presence of CO_2, and peak aggregation in the four centre squares that surrounded the odour source was observed after 24 min (Figure 6). Compared with the control series, a significant increase in the number of individuals that resided in the centre squares was observed after 6 min (Mann-Whitney Rank Sum Test: $T = 515.5$, $P = 0.002$, or $P < 0.001$ for all tests after 6 min; Figure 6). Both males and females moved from the harbourages or border of the arena toward the centre, whereas the control treatments maintained the initial distribution. During peak aggregation at 24 min, females comprised 63% of the animals during the daytime and 44% during the night. The maximum number of bed bugs observed around the CO_2 source during a single stimulation was 12 of 20 individuals. After removal of the CO_2 source, the bed bugs again successively dispersed from the centre of the arena.

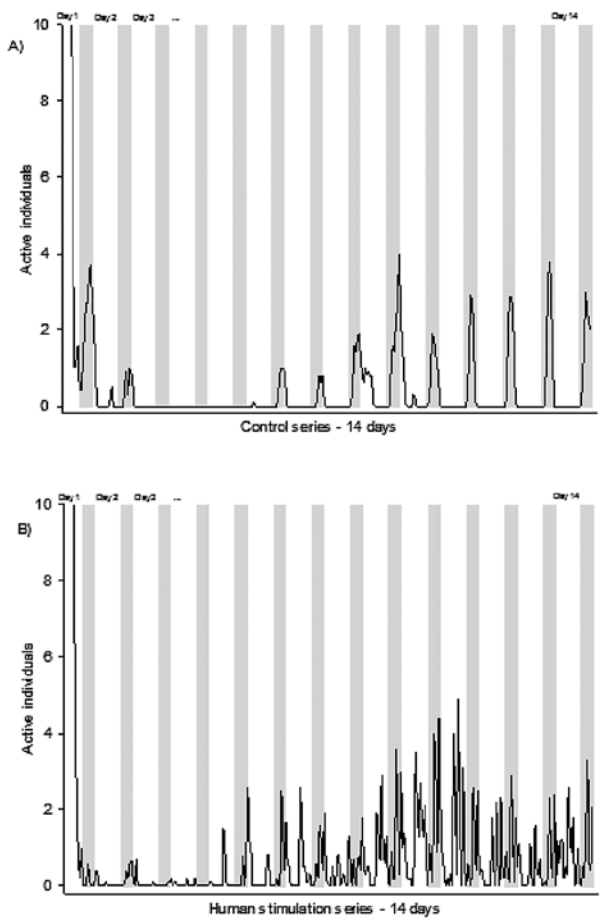

Figure 2. Typical recordings of Cimex lectularius activity in a population of 5 males and 5 females for14 days. (A) Control series without stimuli. (B) Human stimulation series. Gray background = night, white background = lights on.

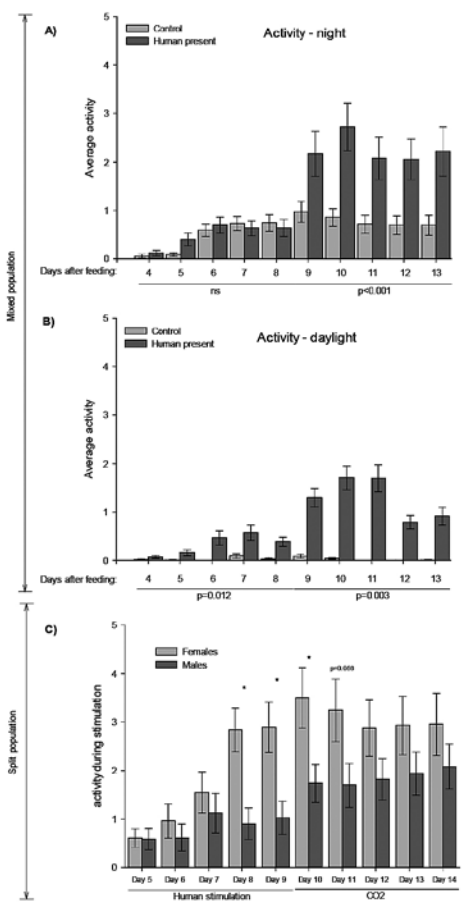

Figure 3. Cimex lectularius activity (average ± SE) during human stimulation of 5 males and 5 females (A) at night and (B) in daylight and (C) during human stimulation from day 5-9 and CO2 stimulation from day 10-14 with 10 males and 10 females in the each half of the arena

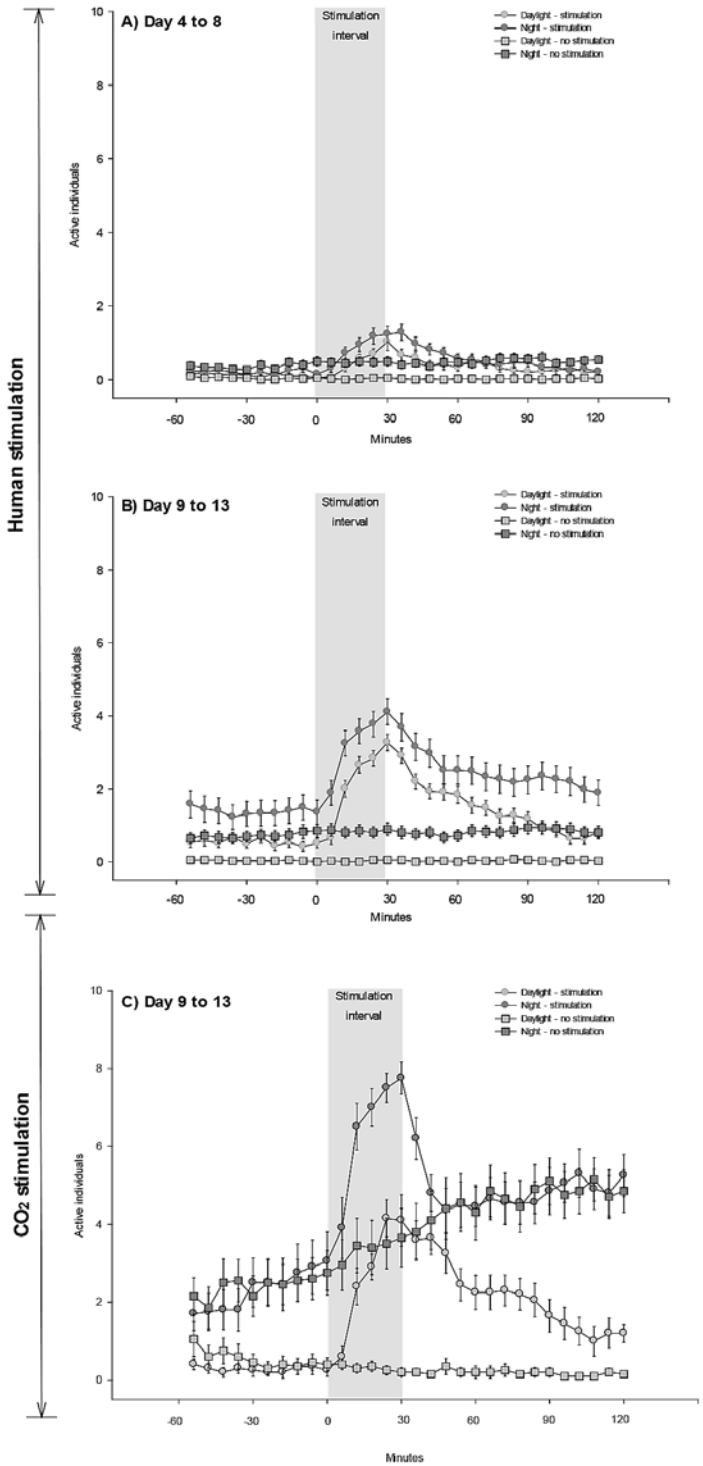

Figure 4. Mean number (± SE) of active Cimex lectularius during stimulation (grey area) with (A) humans on day 4-8 after feeding, (B) humans on day 9-13 after feeding, and (C) CO2 on day 9-13 after feeding.

DISCUSSION

The behaviour observed in the bed bug arena is consistent with bed bug habits that have been previously described in both field and laboratory studies. Activity peaks occur during the night (Romero et al., 2010), with a lack of activity after feeding (Usinger, 1966), modulated movement with the presence

of a host signal (Anderson et al., 2009; Harraca et al., 2012), and only a low level of spontaneous movement in daylight (Romero et al., 2010). These behavioural responses are in agreement with bed bug biology in an urban setting. Our large arena and the use of a 14-day behavioural series appear to mimic natural conditions and may represent a tool for studies that seek to improve field applications in pest control.

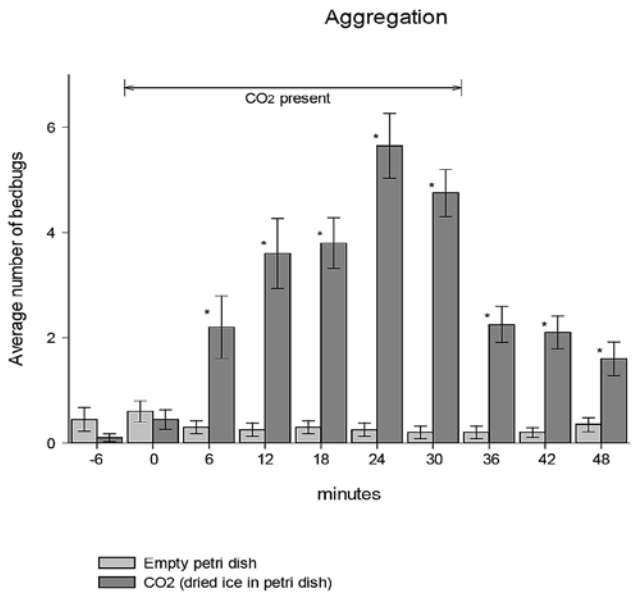

Figure 5. Percentage (± SE) of 10 male and 10 female Cimex lectularius that hid in the corners of the arena during the day and night 6 min prior to stimulation.

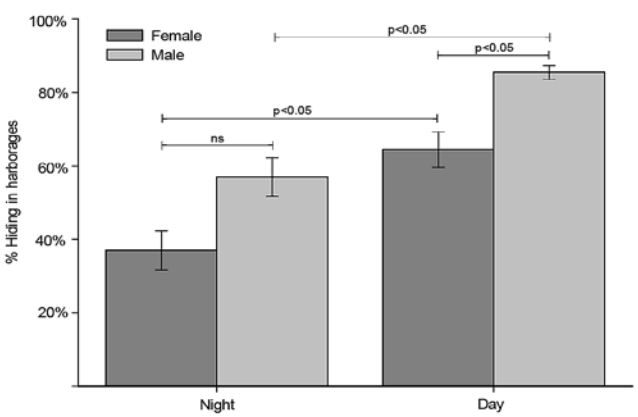

Figure 6. Aggregation of 20 Cimex lectularius (average ± SE) at different times around odour source of dry ice in a Petri dish in middle of arena. Petri dish was removed at 30 min. Empty Petri dish used in the control treatment. *P < 0.05, significant difference in aggregation between stimulation and control.

The present study finds gender differences in host signal responses. Females expose themselves more than males during the day, respond more strongly to the host signal, and respond earlier in terms of the time since feeding. Female egg production will sequester proteins and other elements needed for metabolic processes (Boggs, 2009), and male sperm may be produced and remain viable for a longer time without high metabolic cost. The recorded day-to-day change in the level of responsiveness agrees well with such a nutritional demand scenario because the onset of female activity directed toward a

host appears after resource-draining egg production occurs (after 4-8 days (Usinger, 1966)). The males' gradual increase in activity may be linked to a more fixed metabolic rate. Fitness profit connected to dispersal strategies is an additional explanation of the gender differences. Bed bugs are known to walk long distances in urban environments, and this behaviour occurs mostly in adults (Wang et al., 2010). The sex ratio among dispersing individuals is partially described (Domingue et al., 2010; Pfiester et al., 2009), but the efficient colonization of new habitats by single individuals clearly requires a fertile and inseminated female. Together with the potential location of new habitats, the location of new harbourages for offspring around the present host may be an additional benefit. The distribution of eggs at several locations may be a part of a bet-hedging strategy among females to reduce the total risk of exposure and offspring mortality (Hopper, 1999). Males, in contrast, have no opportunity to recolonize new areas alone and must do this indirectly by mating with females. The chances of finding a new host and a female at the same time may be so low that their optimum strategy is to await mating opportunities in the established nest.

The observed response patterns have implications for the field application of killing agents. As females lay eggs, they are the main target for control and should be the focus of population suppression and eradication (El-Sayed et al., 2006). Most females in the present study remain rather inactive for 5-7 days, even with stimuli present. Such a low level of activity is problematic in terms of exposing bed bugs to killing agents outside their resting places. Because of bed bugs' passivity immediately after feeding (Usinger, 1966), a reasonable strategy will be to leave rooms empty for some days before the application of pesticides or desiccant dusts. This may prevent bed bugs from remaining hidden when the killing agents are most potent. It is not expected that natural populations are as synchronized in feeding as our experimental animals, and frequent and almost continuous feeding and egg laying may occur within a population (Pereira et al., 2013; Reinhardt et al., 2010). Therefore, if artificial host signals are to be utilized efficiently in pest management, then the time since the last feeding and overall hunger state of the population need to be considered. Waiting at least 5 days before applying killing agents may increase the blood lust of a larger proportion of the population to spur the activity of as many individuals during as few stimulation events as possible. Shortening the passive time of the bed bugs might also be possible by raising room temperatures to increase metabolism, the need for a host, and questing activity during artificial stimulation (Reinhardt et al., 2010). Such approaches must be weighed against an increase in egg production and potential hunger-mediated dispersal to adjacent rooms or out of apartments (Wang et al., 2010).

Regardless of when treatment is performed or how it is connected to the feeding habits of the resident population, we demonstrat an effect of CO_2 on the activity level of the bed bugs. Relatively inexpensive and simple activators, such as CO_2 released from dry ice, regulated tanks, or sugar-yeast solutions, can thus be utilized to improve control (Singh et al., 2013). CO_2 is also used to activate bed bugs to make them move across treated substrates (Wang et al., 2013). The present study shows that this single component may be sufficient to manipulate their behaviour to lure them out from their harbourages. The similar response profiles to humans and CO_2 also indicate that CO_2 is the major component of the host signal. The addition of other attractive host compounds (Anderson et al., 2009; Harraca et al., 2012) may further improve such effects. Ideally, all behaviourally active host compounds should be included in the odour replica to lure as many bed bugs as possible into questing and exposing themselves. Currently, full activation can only be achieved with a natural host (i.e., humans), but using natural bait is ethically questionable because many killing agents are toxic to humans, and even residual pesticides may have a negative effect on the health of the

residents (Mostafalou and Abdollahi, 2013). This means that during treatment with potential harmful substances, an artificial host signal is needed to activate the bed bugs to ensure contact with the killing agents. Host signals also appear to more strongly influence egg-laying females, which are an important focus of control efforts.

To identify the full host signal, more behavioural studies are needed, and bioassays that are capable of quantifying bed bug responses should be further developed. Interestingly, in the present study the relative response in terms of activated animals compared with the control condition is largest with the lights on. In daylight, the stimulation produces a peak response with a more than 10-fold increase in activity, whereas activity is only four-times higher at night. Significant differences between human presence and the control condition are also detected earlier (i.e., on day 4-8) only when the lights are on. In terms of measuring differences in odour-released activity, experiments may be performed in the daylight to distinguish small responses from general night-time movements in the arena. Both genders also hide more when experiencing daylight. This facilitates the detection of activity when they leave their harbourages. This rather surprising result may be influenced by the use of habituated laboratory animals that normally feed in the daylight. However, the clear interactions between sex, light setting, and time since feeding should be considered when designing bed bug bioassays.

Arenas appear to be powerful tools for understanding the dynamics of the questing and nesting activity of bed bugs. Our arena contains hiding places. Because the arena is not cleaned between the experimental series, the harbourages likely contain attractive and arresting odours. The human odours are presented in a natural way by simply entering the room, and this triggers a fairly strong and persistent bed bug response. This allows quantification and comparisons with artificial cues. The bed bugs are also found to locate the point source of CO_2 quite efficiently when positioned in the centre of the arena, showing that this single component is sufficient to allow spatial orientation and host location in both daylight and darkness. In addition to providing knowledge for improving control methods, such studies can be used to test, evaluate, and improve the efficiency of traps and lures that are intended to monitor or suppress bed bug populations. The observed behaviour also indicates responses and orientation mechanisms in bed bugs that differ from the more rapid responses and distinct movement patterns found during optomotor anemotaxis in flying insects (Carde and Willis, 2008). The use of more detailed video tracking systems might provide valuable quantification of the behavioural mechanisms that lead to source location in crawling insects that manoeuvre in darkness. Although only an incremental step in elucidating bed bug chemical ecology and the dynamics of questing and nesting activity, we make observations that may contribute to improvements in control strategies. More experimental approaches, combined with field studies, are clearly needed to fully understand the dynamics of a growing bed bug population. Properly revealing the behaviour-regulating features of bed bugs may allow the development of more efficient control methods.

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