Effects of Life Stages and Feeding Regimes on Active Movement Behavior of the Tropical Bed Bug, *Cimex hemipterus* (Hemiptera: Cimicidae)

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ABSTRACT This study examined the effects of different life stages (first, second, third, fourth, and five instars; adult females and adult males) and feeding regimes (starved and blood fed) on the active movement activity of the tropical bed bug, *Cimex hemipterus* (F.), under mixed-stage conditions. We used an extended arena made from Tygon tube coils and observed the movement frequency and movement distance at selected time intervals up to 120 h. The fifth instars and adult males and females showed significantly ($P < 0.01$) greater movement frequency compared with the other stages. The first and second instars showed limited movement (<8 m) over the experimental period. Starved bed bugs showed greater movement frequency compared with blood-fed bed bugs, with the exception of adult females. Blood-fed adult females exhibited significantly ($P < 0.01$) greater movement frequency and distance compared with starved females. Blood-fed females moved up to 42.3 m over 120 h. Regression analysis between movement distance of the fifth instars and adults and the time intervals revealed a positive relationship ($r^2 = 0.6583$; $P < 0.01$), suggesting that delays in bed bug control efforts will increase the risk of the greater infestation. During bed bug inspection, the presence of only late instars and adults in premises would indicate a new infestation, whereas an established infestation likely would consist of mixed stages.

KEY WORDS *Cimex hemipterus*, tropical bed bug, active movement, movement frequency, movement distance

The continuous increase of bed bug infestations in many countries (Krueger 2000; Doggett et al. 2003, 2004; Myles et al. 2003; Hwang et al. 2005; Masetti and Bruschi 2007; Doggett and Russell 2008; Kilpinen et al. 2008; Lee et al. 2008; Potter et al. 2008; How and Lee 2010a) has led to intense research interest. A better understanding of the movement behavior of this increasingly common insect pest is of particular relevance to those trying to manage bed bug infestations. Many studies have discussed the spread of bed bugs via the passive dispersal pathway, through which the insects are carried unnoticed by the host to a new location (Usinger 1966, Paul and Bates 2000, Doggett et al. 2003, Reinhardt and Siva-Jothy 2007, Kilpinen et al. 2008). Bed bugs, however, can also actively disperse over relatively shorter distances, such as from room to room within a building and between contiguous buildings (Reinhardt and Siva-Jothy 2007, How and Lee 2010a, Wang et al. 2010). Because the spread of bed bugs is often random and spontaneous (Doggett and Russell 2008), it is crucial to understand both passive and active dispersal behaviors.

In this study, we examined the effects of different life stages and feeding regimes on the active movement behavior of the tropical bed bug, *Cimex hemipterus* (F.). The study of active bed bug movement in the field presents numerous logistical and technical challenges, especially regarding movement distance, and so a laboratory model was developed. Using the laboratory model, we were able to measure movement frequency (the percentage of total number of observed movements of bed bugs in the experimental arena during the experimental period) and movement distance to quantify the movement of bed bugs. The experiments were conducted under mixed-stage conditions. Our results illustrate the potential movement behavior of *C. hemipterus* under experimental conditions, and these results provide practical information for bed bug inspection and management.

Materials and Methods

Insect Samples and Experimental Conditions. We used field-collected populations of *C. hemipterus* that have been cultured in the laboratory since 2006. The strains used were the KMelayu14 strain (collected from Penang Island, Malaysia) and the Serangoon

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Fig. 1. The modified extended arena for studying *C. hemipterus* active movement activity: (A) point of introduction (glass vial) and (B) point of termination (plastic container).

strain (collected from Singapore) (How and Lee 2010a). Bed bugs were reared in glass jars (7 cm diameter × 9 cm height) with folded brown paper as harborages under environmental conditions of 26 ± 2°C, 70 ± 5% RH, and a 12-h photoperiod. All bed bugs used in this study were fed with fresh human blood from two volunteers. Adults (males and females) used in this study were assumed to have mated, as they were not sex segregated.

Experimental Arena. The extended arena (20 m) used to measure bed bug movement was modified after Su's (2005) method for studying termite foraging. It consisted of five 4-m-long coiled 0.32-cm-diameter Tygon tubes (Saint-Gobain Performance Plastics, Seattle, WA). Each coil was joined and sealed with parafilm-M (Pechiney Plastic Packaging, Chicago, IL). The coiled tube was fixed tightly on a polyethylene tray (48 cm length × 36.5 cm width × 8 cm height), and the surface of its inner wall was lined with fluon to prevent bed bugs from escaping (Fig. 1). The test insects were placed in a glass vial that was connected to the introduction point (A), as shown in Fig. 1. The terminal end of the extended arena was connected to a container (B). The testing tray was covered by another tray in complete darkness, and it was uncovered for only several minutes at a time to collect data. To exclude the effects of any aggregation pheromone, the glass vials were soaked overnight and washed using a cleaning agent (Biorex M; Glaswarenfabrik Karl Hecht, Sondheim-Rhön, Germany) before being reused, and only new tubes were used for each replicate.

Movement Frequency and Distance. Movement frequency refers to the mean percentage of the number of bed bugs of each stage that were observed to have moved in the extended arena during the entire experimental period over the total number of bed bugs in all data-taking intervals ($n = 70$). Data were taken at the following time intervals: 6, 12, 24, 48, 72, 96, and 120 h after the experiment was started. When movement was detected, the location of the insect on the Tygon tube was marked; if the bed bug had advanced or retreated at the subsequent time point, a movement was indicated.

Movement distance refers to the mean distance (in meters) traveled by the bed bug of each stage in the extended arena during the experimental period. We

Table 1. Mean cumulative age of the different life stages of *C. hemipterus*

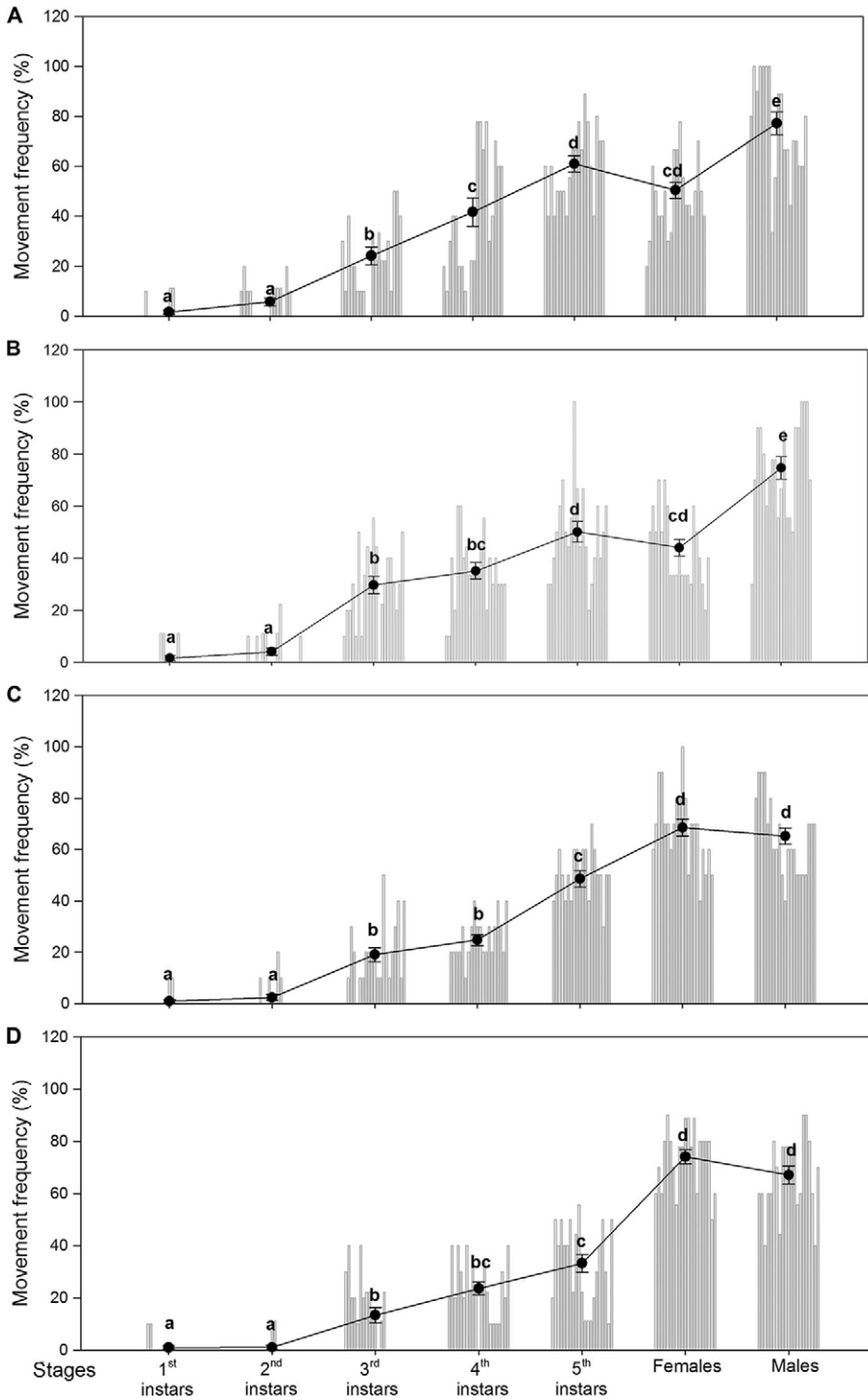| Strain	Stage	Mean cumulative age (d)
KMelayu14	First instars	6.57
	Second instars	9.95
	Third instars	13.08
	Fourth instars	16.25
	Fifth instars	19.84
	Adult (male and female)	24.62
Serangoon	First instars	6.14
	Second instars	9.50
	Third instars	13.01
	Fourth instars	16.67
	Fifth instars	20.08
	Adult (male and female)	24.47

Fig. 2. Distribution of active movement frequency of the various life stages of *C. hemipterus* in a mixed-stage experiment. (A) Starved KMelayu14 strain, (B) starved Serangoon strain, (C) blood-fed KMelayu14 strain, and (D) blood-fed Serangoon strain. Different letters across various stages indicate significant differences (Fisher LSD, $P < 0.01$).

measured the distance the bed bug traveled (both forward and reverse distances) by marking the location of the insect at each time point as compared with the previous time point. The coil was straightened and the movement distance was measured with a ruler. The cumulative movement distance was calcu-

lated using the following formula: (1st ML + [2nd − 1st]ML + [3rd − 2nd]ML + . . .), where ML = marked length on the tubing.

Effects of Stages and Feeding Conditions. We tested *C. hemipterus* in mixed-stage groups to determine the effects of life stages and feeding conditions on movement frequency and distance. For the mixed-stage experiment, one blood-fed individual of each (first, second, third, fourth, and fifth) instar along with an adult male and female were placed into a glass vial at the introduction point. These bed bugs were given a blood meal within 24–48 h before the start of the experiment. A total of 30 replicates was performed, and then the experiment was repeated with unfed bugs (a blood meal was given 7 d before the start of the experiment). Both starved and blood-fed bed bugs were isolated from laboratory cultures and preconditioned inside the glass vials for a few hours before the experiment began.

Data Analysis. Movement frequency (after arcsine square-root transformation) and movement distance data were subjected to analysis of variance, and means were separated using Fisher least significant difference (LSD). The correlation between movement frequency and mean cumulative age of the different stages was determined using the Spearman rank test, and correlation between movement distance and age of the different stages was assessed using Pearson correlation. Table 1 lists the cumulative ages of the different life stages of bed bugs. Comparison of movement frequency and distance between starved and blood-fed bed bugs and between different life stages was performed using Student's *t* test. The relationship between potential movement distance and time intervals for the three most active stages of *C. hemipterus* in the mixed-stage experiment was determined using linear regression. All analyses were conducted using SPSS version 12.0.1 (SPSS 2003).

Results

Movement Frequency. Bed bugs of different stages differed significantly ($F_A = 50.86$, $F_B = 50.75$, $F_C = 89.65$, $F_D = 61.90$; df = 6, 140; $P < 0.01$) in movement frequency and generally could be separated into three groups (Fig. 2). The first group consisted of the early nymphs (first and second instars), which were the least active stage; they spent almost 80% of the time at rest. The second group consisted of the middle-stage nymphs (third and fourth instars), and the third group consisted of late instars and adult males and females. The observed difference between the second and third groups was less profound than the differences between them and the first group. The males were the most active stage, especially when starved. The blood-fed females were more active than the starved females. The Spearman rank test indicated that bed bugs (starved and blood fed) became more active with increasing life stage (Table 2).

Starved bed bugs in the mixed-stage experiment showed significantly greater movement frequency ($t_{\text{second instars}} = 2.46$, $t_{\text{third instars}} = 3.50$, $t_{\text{fourth instars}} =$

Table 2. Correlation between movement parameters and mean cumulative age of different life stages of *C. hemipterus*

Parameter	Strain	Condition	Correlation coefficient[a]
DF	KMelayu14	Starved	$r_s = 0.880$
		Fed	$r_s = 0.965$
	Serangoon	Starved	$r_s = 0.903$
		Fed	$r_s = 0.929$
DD	KMelayu14	Starved	$r = 0.824$
		Fed	$r = 0.804$
	Serangoon	Starved	$r = 0.857$
		Fed	$r = 0.841$

DF, movement frequency; DD, movement distance; r_s, Spearman rank correlation; r, Pearson correlation.
[a] All correlations were significant at $P < 0.01$.

3.79, $t_{\text{fifth instars}} = 4.01$, $t_{\text{male}} = 3.16$; df = 82; $P < 0.01$) than those that were blood fed, with the following exceptions: the first instars did not show any significant difference between the two feeding regimes ($t = 0.81$, df = 82, $P > 0.05$), and movement frequency of blood-fed adult females was significantly greater than that of starved adult females ($t = -7.49$, df = 82, $P < 0.01$).

Movement Distance. We observed frequent retracing movements of bed bugs during testing, regardless of life stage or feeding status. Bed bugs moved forward and retreated toward the glass vial from time to time. The movement distance is the summation of the total distance traveled during the duration of the experiment. Significant differences in movement distance were detected among various life stages and feeding regimes in the experiment ($F_A = 15.25$, $F_B = 18.25$, $F_C = 20.81$, $F_D = 50.99$; df = 6, 203; $P < 0.01$) (Fig. 3). Pearson correlation ($r > 0.8$) showed that movement distance significantly ($P < 0.01$) increased with increasing life stage (Table 2). The fifth instars were able to move up to 17.0–41.2 m and adult insects could travel up to 23.4–42.3 m after 120 h. In contrast, the early instars (first and second instars) showed less movement (<8 m) during the course of the experiment.

Blood-fed females showed significantly ($t = -3.88$, df = 118, $P < 0.01$) greater movement distance than starved females. The other stages showed no significant difference ($P > 0.05$) in movement distance between blood-fed and starved individuals. Throughout the 120-h observation of movement activity, no mortality was recorded, and a total of eight blood-fed and four starved bed bugs (fifth instars, adult males, and females) successfully reached end point B (Fig. 1).

Relationship Between Movement Distance and Time. Regression analysis between movement distance of the three most active stages and time intervals showed a significant positive relationship ($r^2 = 0.6583$, $s = 6.2220$, $P < 0.01$) (Fig. 4). This result could be used as a reference for potential movement of bed bugs in nature and potentially could be applied during bed bug inspections.
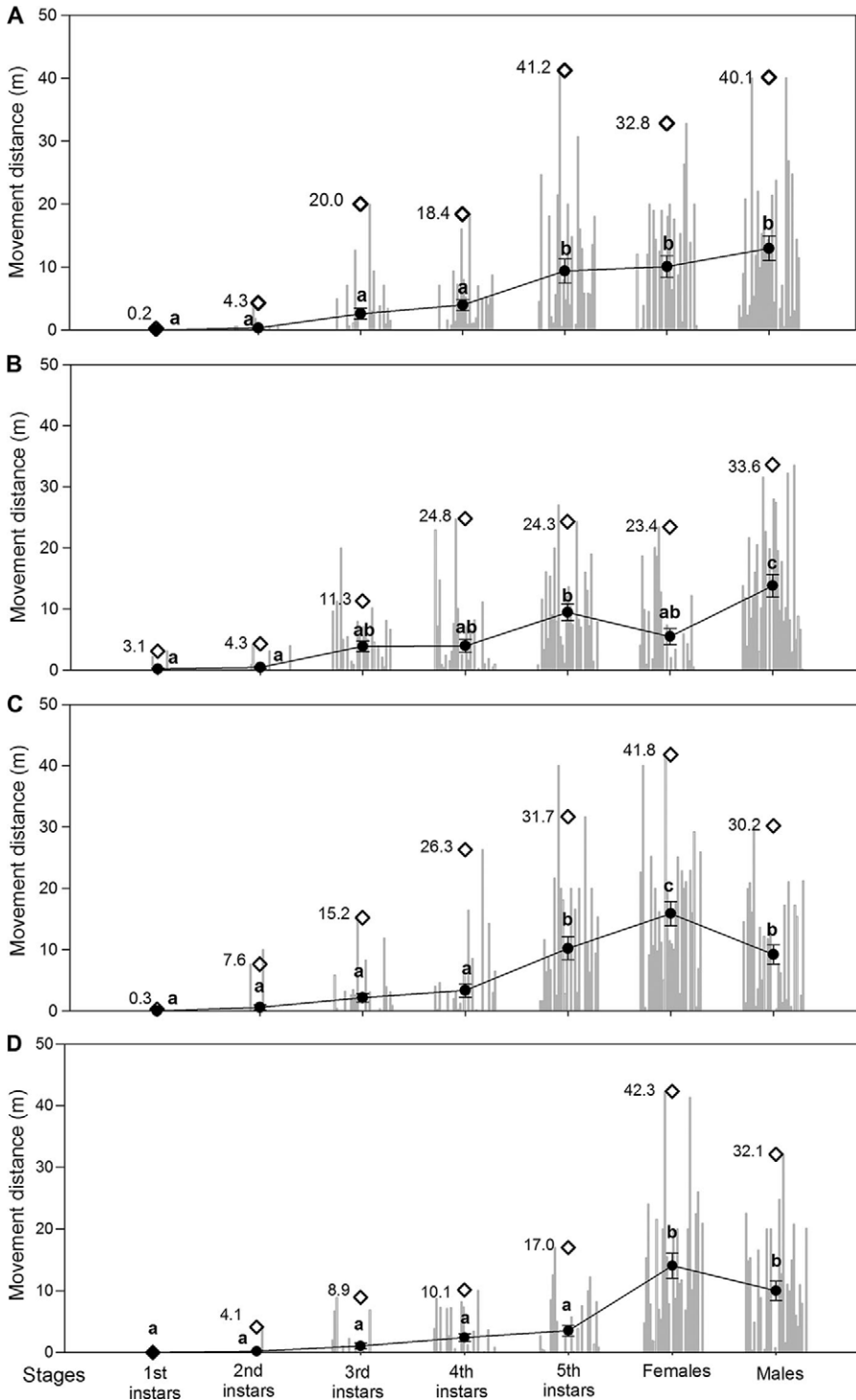
Fig. 3. Distribution of active movement distance of various life stages of *C. hemipterus* in a mixed-stage experiment. (A) Starved KMelayu14 strain, (B) starved Serangoon strain, (C) blood-fed KMelayu14 strain, and (D) blood-fed Serangoon strain. Different letters across various stages indicate significant differences (Fisher LSD, $P < 0.01$). The ◇ symbol with a number represents the modal value of observed movement distance.
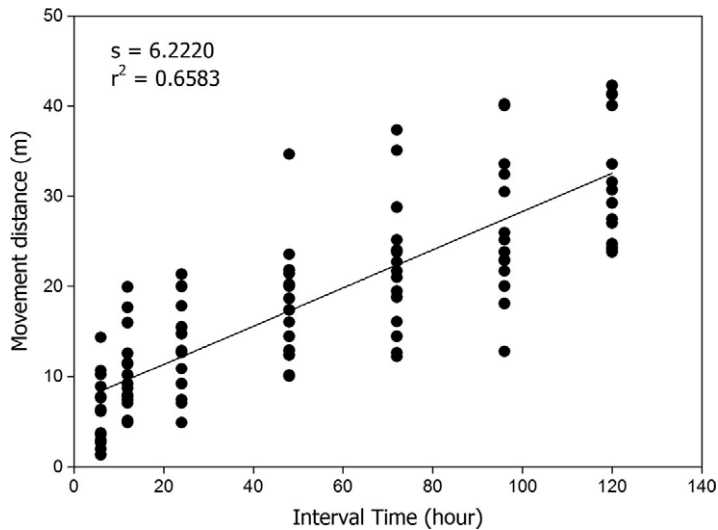
Fig. 4. Linear regression of the relationship between movement distance and time intervals for the three most active stages (starved fifth instars, starved adult males, and blood-fed females) of *C. hemipterus* in a mixed-stage experiment; $y = 7.12 + 0.21x$, where $y =$ self-movement distance and $x =$ interval time in hours.

Discussion

The fifth instars and adult *C. hemipterus* were the most active stages, showing greater movement compared with the other stages. Moreover, blood-fed adult females were more active than those that were starved. This observation was in agreement with that made by Pfiester et al. (2009a) for *Cimex lectularius* L. In our study, the early instars moved very little during the course of the experiment, and some had not even crawled out from the glass vial at the introduction point. This behavior reflects their preference to aggregate, as reported by Pfiester et al. (2009a). They found that 94–98% of *C. lectularius* early instars spent most of their time in aggregation, which likely was promoted by the presence of contact and airborne aggregation pheromones (Siljander et al. 2007, 2008; Olson et al. 2009). In comparison, we found fifth instars up to 41.2 m from the introduction point, and movement frequency ranged from 40 to 80% for this stage.

In this study, fifth instars, adult males, and adult females of *C. hemipterus* showed the greatest movement activity, and first and second instars were the least active. Wang et al. (2010) found higher movement numbers of adults compared with nymphs in interceptor traps. Kemper (1936), Usinger (1966), and Siljander et al. (2007) reported that *C. lectularius* responded to adult- and juvenile-specific contact aggregation pheromones and aggregated in mixed-stage colonies. In the field, bed bugs are commonly found in mixed-stage populations (Johnson 1941, Reinhardt and Siva-Jothy 2007). Thus, the presence of only fifth instars and adults in a premises would indicate that the infestation likely is new, whereas the presence of mixed stages would suggest that the infestation has been established in the premises for a period of time.

The physiological condition (i.e., starved or blood fed) of bed bugs affects their movement behavior. Reinhardt and Siva-Jothy (2007) and Olson et al. (2009) both stated that aggregation behavior is negatively correlated with starvation duration. These reports support our observation that most of the starved stages showed greater movement frequency than those that received a blood meal. Hunger increases movement of insects from their harborage sites, and this may also promote formation of new harborage sites (Griffiths 1980, Bell 1990). In addition to hunger, mate finding is another factor that encourages dispersal movements, particularly in adult males (Pfiester et al. 2009a).

Mellanby (1939) and Johnson (1941) reported that females were often more active than males in active dispersal. In the current study, this was true only for blood-fed females, which were more active than starved females. Blood-fed females with an extended abdomen would better attract males engaged in mate-searching activity (Pfiester et al. 2009b, Reinhardt et al. 2009). We found that blood-fed females dispersed at higher frequency and at a greater distance, perhaps to reduce the chances of repeated insemination by males (Stutt and Siva-Jothy 2001, Morrow and Arnqvist 2003, Reinhardt et al. 2003, Siva-Jothy 2006). Repeated traumatic insemination is costly to females because of multiple abdominal piercing wounds and reduced life span (Morrow and Arnqvist 2003, Reinhardt et al. 2003, Pfiester et al. 2009b, How and Lee 2010b). Siljander et al. (2008) reported that female bed bugs were likely to disperse singly in the field. This report reinforced our hypothesis that blood-fed females were more active to avoid repeated insemination. In addition, blood-fed females likely increased their searching behavior to look for harborage and

oviposition sites (Hassell and Southwood 1978, Bell 1990).

Active movement is likely to be an important mode of bed bug infestation from room to room within a building and between contiguous buildings (Reinhardt and Siva-Jothy 2007, How and Lee 2010a, Wang et al. 2010). Our study revealed that even in the absence of a host, *C. hemipterus* can travel up to 42.3 m after 120 h. This indicates that an infestation can potentially spread from one room to another over several days. However, linear movement may be affected by several factors in natural bed bug infestations, which could cause bed bugs to migrate shorter or farther distances. Factors other than life stage and feeding status that may influence the movement of bed bugs include disturbance by repellent insecticides (Romero et al. 2009), temperature (Bell 1990), population overgrowth (Wertheim et al. 2005), and the presence of a host (Potter et al. 2008).

Acknowledgments

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