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Effects of Temperature and Humidity on the Survival and Water Loss of *Cimex hemipterus* (Hemiptera: Cimicidae)

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ABSTRACT The effect of temperature and humidity on the survival and water loss of the tropical bed bug, *Cimex hemipterus* (F.), was studied using two field-collected strains. Insects were exposed to temperatures ranging from 20 to 45°C and relative humidities (RHs) of 33, 75, and 100%. *C. hemipterus* survived longest under the interaction of low temperature (20°C) and high RH (75–100%). Survival and water loss were significantly affected ($P < 0.01$) by temperature and RH (either singly, or in interaction). Strain and sex significantly ($P < 0.01$) influenced bed bug survival, but not on water loss. Eggs, first instars, and adults reached their upper thermal lethal limit within 1 h at 39°C, 44°C, and 46°C, respectively. The survival and water loss profiles showed that starved *C. hemipterus* started to die after losing 35–45% of their body weights.

KEY WORDS *Cimex hemipterus*, temperature, humidity, survival, water loss

Bed bugs are becoming an increasingly important urban insect pest worldwide based on the rising number of infestations (Krueger 2000, Boase 2004, Doggett et al. 2004, Potter 2006, How and Lee 2010a). Bed bug bites cause insomnia because of itchiness, allergic hypersensitivity, hemoglobin iron deficiency, and cutaneous weal formation, and they may even trigger a secondary infection (Venkatachalam and Belavady 1962, Feingold et al. 1968, Jupp et al. 1991, Blow et al. 2001, Goddard and deShazo 2009, Kolb et al. 2009).

Few studies have been conducted to evaluate the environmental factors that influence the two anthropophilic species of bed bugs: the bed bug, *Cimex lectularius* L. (Mellanby 1935; Johnson 1940a, 1940b, 1941; Omori 1941; Usinger 1966; Benoit et al. 2007), and the tropical bed bug, *Cimex hemipterus* (F.) (Mellanby 1935, Omori 1941, Usinger 1966). A resurgence of bed bug infestations after almost 60 yr of quiescence has renewed interest in the biology and toxicology of these insects. Survival and thermal-tolerance limits are two of the most critical biological factors for bed bug control purposes. Survival and thermal-tolerance limits also can be used to provide useful information for management of bed bugs via heat treatment (Roberto et al. 2009).

In our study, we examined the survival of *Cimex hemipterus* under different regimes of temperature and relative humidity (RH). To date, the interaction of both environmental factors has not been thor-

oughly investigated. Although the water balance profile of the common bed bug and its tolerance to dehydration have been examined (Benoit et al. 2007), our research expanded existing knowledge by building a survival and water loss profile of the tropical bed bug based on the effects of the interactions between temperature and RH.

Materials and Methods

Insects and Experimental Conditions. We used field-collected populations of *C. hemipterus* that have been reared in the laboratory since 2006. They were kept in glass jars (7 cm diameter × 9 cm height) with folded brown paper as harboring sites under the environmental conditions of $27 \pm 2^\circ\text{C}$, $70 \pm 5\%$ RH, and a 12-h photoperiod. Two strains were used, as follows: KMelayu5 from Malaysia and Soon Lee from Singapore (How and Lee 2010a). All bed bugs used in this study were fed using a human volunteer. Mated adults were obtained from mixed cultures of males and females. Unmated adult males and females were segregated soon after the final molt (before sclerotization). The males and females were kept in separate containers before experiment. The adults were blood fed once ≈ 4 –5 d before the experiment began to minimize the physiological effects of excretion, digestion, and depletion of blood (Benoit et al. 2007). The adults were aged 7–10 d at initiation of the experiment. No blood feeding was provided to the insects during the course of experiment.

Tests involving temperature of 20–35°C were carried out using a Venticell incubator (Medcenter Ein-

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richtungen GmbH, München, Germany), whereas tests involving temperature $>35^{\circ}\text{C}$ were run on a digital oven (Mettler, 854 Schwabach, Germany). The internal temperature of the incubator and oven was calibrated and measured by a thermometer to maintain and ensure the temperature deviation of less than $\pm 1^{\circ}\text{C}$ (mean deviation within $\pm 0.5^{\circ}\text{C}$) before the initiation of the experiment. RH was maintained using saturated salt solutions, as follows: 33% (MgCl_2), 75% (NaCl), and 100% (pure distilled water) (Winston and Bates 1960). All experiments were conducted in an air-tight polyethylene container ($17 \times 11.5 \times 6.5$ cm) in which the insects were kept in glass vials (diameter 2.5 cm, height 5 cm) and the saturated salt solution was kept in a glass jar. The experiments were carried out in clusters using factorial design with three to six replicates of five to 10 insects per replicate, which were kept in the same air-tight container and then introduced into the above-mentioned temperature-controlled devices.

Upper Thermal Lethal Limit. The upper thermal lethal limits of insect eggs, first instars, and adults of two strains were determined by subjecting each stage to temperatures at 1°C increments (adjusted and calibrated before the test) starting from 35, 42, and 45°C , respectively. These insects were subjected to 1-h exposure at each temperature, until 100% mortality was observed. The 1-h exposure was chosen so that the results would be compared with those reported earlier by Mellanby (1935), Usinger (1966), and Benoit et al. (2009). Mortality was recorded after the exposure period. If no complete mortality was observed, the exposure would be prolonged up to 24 h. During this period, mortality of the eggs and insects was examined at selected time intervals.

Survival Under Interactions of Temperature and Humidity. The experiment was conducted at five different temperatures (20°C , 27°C , 35°C , 40°C , and 45°C), and each temperature was tested at three RHs (33, 75, and 100% RH). We used unfed first instars (within 24 h after hatching) and adults aged 3–5 d (both mated and unmated) from the Soon Lee and KMelayu5 strains to determine their survival period. All insects and eggs were observed daily for mortality and nymphal emergence.

We transformed the RH value into vapor pressure deficit (in millibars) using the following formula (Ward and Trimble 2004) to illustrate the interactions of survival and humidity under different temperatures and RHs through univariate test: vapor pressure deficit = $e_s - e_d$, where e_s is the saturation vapor pressure at air temperature (millibars), and e_s can be computed as follows: $e_s = \exp([16.78 T - 116.9] / [T + 237.3]) \times 10$, where T = temperature in $^{\circ}\text{C}$; e_d is the actual vapor pressure (millibars) and can be computed as follows: $e_d = (e_s \times \text{RH}) / 100$.

Water Loss Under Interactions of Temperature and Humidity. This experiment was conducted using the same life stage of insects in the above-mentioned survival study. The bed bug water loss in relation to temperature and RH was recorded at 1, 4, 8, 12, 24, and 48 h. The average weight of the experimental bed bugs

(five to 10 insects per replicate, three replicates) was measured using an electronic analytical balance (Sartorius BP 190S, Goettingen, Germany). As all bed bugs were similar in size, the weight variation between each individual was assumed to be negligible. Hence, % water loss = % weight loss = $([\text{initial weight} - \text{weight after exposure}] / \text{initial weight}) \times 100$.

This calculation is based on the assumption that weight loss was the result of incidental and respiratory water loss and not related to other effects (Gunn 1933, Kemper 1936, Klok and Chown 1997). Thus, water loss can be directly calculated from the mass recorded at the various time intervals in conjunction with the set temperature and RH. Throughout the experiment, water may also be lost through defecation, especially after a blood meal (Benoit et al. 2007). We minimized this possibility by using the bugs that were blood fed 4–5 d before the experiment, and no blood meal was given during the experiment.

Statistical Analysis. One-way analysis of variance was used to compare the longevity of survival and egg hatchability of different life stages and strains ($P = 0.05$). The relationship between the upper thermal lethal limit and survival time (after log transformation) was analyzed using linear regression. The interactions between various extrinsic factors (temperature and RH) and intrinsic factors (sex and strain) and their effects on survival were analyzed using the univariate test ($P = 0.01$), followed by stepwise regression. The same univariate test also was applied on water loss ($P = 0.01$). Tukey's honestly significant difference test was used for post hoc comparison. Differences in survival between mated and unmated adults, between unmated adults and first instars, and between mated adults and first instars were determined using t test ($P = 0.05$). All statistical analyses were performed using SPSS 2003 version 12.0.1 for Windows (SPSS, Chicago, IL).

Results and Discussion

Upper Thermal Lethal Limit. The three most critical life stages of bed bugs (egg, first instars, and adult) were used in this study. Adult stages are important in bed bug control, as they are capable of reproduction and long distance movement (How and Lee 2010b, 2010c). The eggs are the most tolerant stage to chemical control (Krueger 2000, Gunderson and Strand 1939), and the eggs will hatch into first instars that are the most fragile and sensitive stage (Omori 1941, Benoit et al. 2007).

In the upper thermal lethal limit test, percentage of egg hatchability decreased significantly as temperature increased, beginning with temperature of 37°C . Between 90 and 100% of all eggs hatched at 20, 27, and 35°C . The upper thermal lethal limit of the eggs was reached at 38°C and 39°C for KMelayu5 and Soon Lee strain, respectively, in which all eggs failed to hatch (Table 1). The egg incubation period shortened as the temperature increased, with mean ranging between 6 and 9 d (maximum 12 d) for 20°C , 4 and 6 d (maximum 7 d) for 27°C , and 3 and 4 d (maximum 5 d) for 35°C .

Table 1. Influence of various temperatures at 75% RH on the egg incubation period (in days) and percentage of egg hatchability of *C. hemipterus*

Strain	n		Response at various temperatures ^a					
			20°C	27°C	35°C	37°C	38°C	39°C
KMelayu5	30	Incubation period (mean day ± SE)	8.9 ± 0.4a	5.8 ± 0.2b	3.8 ± 0.1c	2.4 ± 0.1d	2.2 ± 0.2d	0.0 ± 0.0
		% hatchability	90.0	100.0	100.0	53.3	16.7	0.0
Soon Lee	30	Incubation period (mean day ± SE)	6.2 ± 0.2a	4.4 ± 0.3b	3.9 ± 0.1b	2.5 ± 0.2c	0.0 ± 0.0	0.0 ± 0.0
		% hatchability	100.0	100.0	100.0	33.3	0.0	0.0

^a Means followed by the different letters within the same row are significantly different ($P < 0.05$, Tukey's honestly significant difference test).

At higher temperature of 37°C for eggs that survived the treatment showed incubation period of only 2–3 d (maximum 3 d) for Soon Lee strain, whereas KMelayu5 strain registered the same results at 38°C.

Previous studies of Mellanby (1935) and Johnson (1940a) reported that no egg hatched at 37°C for both *C. lectularius* and *C. hemipterus*. However, at this temperature, we observed a 33–54% egg hatchability rate in both strains. In our study, 16.7% of all eggs of the KMelayu5 strain survived up to 38°C, whereas all eggs of Soon Lee strain perished within 24 h at 38°C.

Table 2 showed that the first instars encountered their upper thermal lethal limit at 44°C. Under the influence of increased temperature, survival of first instars decreased by half in ≈10–25 d across 20–27°C. In addition, the first instars exposed to 35°C survived a mean range of 6.9–7.6 d (maximum 9 d). However, Mellanby (1935) stated that first instars survived for only a mean range of 2.7–4.5 d at 36°C under a wide range of RHs (0–90%).

In this study, the upper thermal lethal limit at 1 h (=0.042 d) of exposure for adults was >45°C. All adults were killed within 0.042 d and 0.083 d after exposures at 46°C and 45°C, respectively. This was 1–2°C higher than that reported by Mellanby (1935) and Usinger (1966). All adults of *C. hemipterus* were unable to survive for 24 h of exposure to 40°C.

The relationship between the upper thermal lethal limit (temperature) of adult *C. hemipterus* and the length of survival was determined by plotting the log of the bed bug survival time in hours against temperature and calculating a best-fit line (Fig. 1). The regression was significant ($r^2 = 0.993$, $s = 0.079$, $F =$

8174.83, $P < 0.001$), and the equation obtained was as follows: $\log_{10}y = 9.633 - 0.208x$, where y = survival time (h) and x = upper thermal lethal limit (°C). The best-fit line indicated that as temperature increased, there was an exponential decrease in bed bug survival time. However, this relationship is restricted only to the temperature range of the upper thermal lethal limit of 35–46°C.

The upper thermal lethal limit of *C. hemipterus* was higher than that of *C. lectularius*. Roberto et al. (2009) indicated that adult *C. lectularius* began to die at temperatures >39°C, and 100% mortality was achieved at 43°C for 25 min. Species from warmer habitats have a higher upper thermal lethal limit compared with species from cooler climates (Cohen and Cohen 1981). *C. lectularius* is a temperate species, whereas *C. hemipterus* is subtropical and tropical in nature. However, the potential for preconditioning or acclimatization may result in the overlapping of their geographical distributions. This supposition is supported by an observation from Kwazulu, South Africa, where hybrids of *C. hemipterus* and *C. lectularius* were found (Newberry 1988, 1989; Walpole and Newberry 1988). Doggett et al. (2003) also collected both species of *Cimex* in Australia.

The temperature tolerance of insects could be influenced by a number of factors (Chapman 1998) that included temperature hardening and dehydration level of bed bugs, *C. lectularius* (Benoit et al. 2009). Benoit et al. (2009) have shown that *C. lectularius* that had been held for rapid heat hardening (acclimatized at 37°C for 1 h before heat exposure) could sustain up to 48°C after 1-h exposure with <5% survival. The

Table 2. Influence of various temperatures at 75% RH on the survival time (in days) of first instars, and adults of *C. hemipterus*

Strain	Stage	n	Response at various temperatures ^a (mean day ± SE)								
			20°C	27°C	35°C	40°C	42°C	43°C	44°C	45°C	46°C
KMelayu5	First instars	30	36.3 ± 1.1a	26.1 ± 0.8b	7.6 ± 0.2c	NA	0.1 ± 0.0d	0.1 ± 0.0d	0.0 ± 0.0	NA	NA
	Mated adult ♂	15	27.6 ± 3.1a	22.4 ± 1.6a	3.0 ± 0.6b	0.6 ± 0.1b	NA	NA	NA	0.1 ± 0.0b	0.0 ± 0.0
	Mated adult ♀	15	30.0 ± 1.6a	27.8 ± 1.5a	6.2 ± 0.8b	0.6 ± 0.1c	NA	NA	NA	0.1 ± 0.0c	0.0 ± 0.0
	Unmated adult ♂	15	50.1 ± 2.6a	32.0 ± 2.9b	4.9 ± 0.6c	0.8 ± 0.1c	NA	NA	NA	0.1 ± 0.0c	0.0 ± 0.0
	Unmated adult ♀	15	101.1 ± 6.2a	62.4 ± 3.8b	5.8 ± 0.9c	0.6 ± 0.1c	NA	NA	NA	0.1 ± 0.0c	0.0 ± 0.0
Soon Lee	First instars	30	48.1 ± 1.1a	23.5 ± 0.8b	6.9 ± 0.3c	NA	0.1 ± 0.0d	0.1 ± 0.0d	0.0 ± 0.0	NA	NA
	Mated adult ♂	15	33.4 ± 2.8a	24.0 ± 2.4b	4.6 ± 0.7c	0.8 ± 0.1c	NA	NA	NA	0.1 ± 0.0c	0.0 ± 0.0
	Mated adult ♀	15	29.3 ± 2.3a	19.5 ± 1.5b	5.4 ± 0.7c	0.9 ± 0.1cd	NA	NA	NA	0.1 ± 0.0d	0.0 ± 0.0
	Unmated adult ♂	15	72.5 ± 4.7a	59.8 ± 3.1b	6.2 ± 0.8c	0.8 ± 0.1c	NA	NA	NA	0.1 ± 0.0c	0.0 ± 0.0
	Unmated adult ♀	15	98.6 ± 6.6a	74.5 ± 5.4b	5.8 ± 1.0c	0.8 ± 0.1c	NA	NA	NA	0.1 ± 0.0c	0.0 ± 0.0

^a Means followed by the different letters within the same row are significantly different ($P < 0.05$, Tukey's honestly significant difference test). NA, data not available.

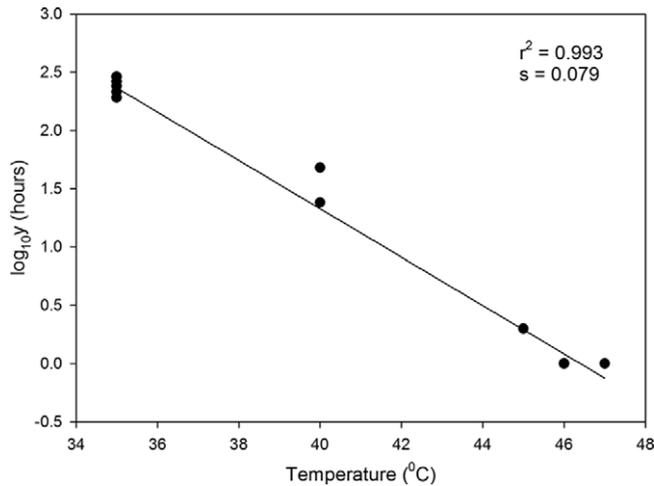


Fig. 1. Linear regression showing the relationship between the upper thermal lethal limit and the length of survival of male and female adult bed bugs (*C. hemipterus*) for various temperatures ($n = 60$).

authors also suggested the expression of two heat shock proteins, Hsp70 and Hsp90 in *C. lectularius*, as response to both heat and cold stresses. These heat shock proteins were also found in many other insects orders, including Diptera, Lepidoptera, Coleoptera, and Hymenoptera (Gehring and Wehner 1995, Rinehart et al. 2007, Elekonich 2009).

Survival Under Interactions of Temperature and Humidity. The survival of mated adults (males and females) decreased in relation to increasing temperature as other stages do (Table 1). The decline in survival was obvious as temperatures rose from 27 to 40°C (Fig. 1). These results are similar to those reported by Benoit et al. (2007). Compared between the two groups, the first instars survived longer ($df = 58, P < 0.05$) than mated adults at both lower (20°C) ($t_{KMelayu5} = 3.70, t_{Soon Lee} = 7.96$) and higher temperatures (35°C) ($t_{KMelayu5} = 4.90, t_{Soon Lee} = 3.35$) (Fig. 2). In contrast, unmated adults survived longer ($df = 58, P < 0.05$) than the first instars at 20°C ($t_{KMelayu5} = 6.69, t_{Soon Lee} = 6.47$) and 27°C ($t_{KMelayu5} = 4.22, t_{Soon Lee} = 12.65$) (Fig. 3).

Experiment ($n = 15$) on survival between mated and unmated adults (Fig. 4) under five different temperatures (20°C, 27°C, 35°C, 40°C, and 45°C) at 75% RH revealed no significant difference ($df = 28, t_{KMelayu5, \diamond, 35^\circ C} = 1.75, t_{KMelayu5, \diamond, 40^\circ C} = 1.58, t_{KMelayu5, \diamond, 45^\circ C} = 0.00, t_{KMelayu5, \square, 5^\circ C} = 0.33, t_{KMelayu5, \square, 40^\circ C} = 0.00, t_{KMelayu5, \square, 45^\circ C} = 0.36, t_{Soon Lee, \diamond, 35^\circ C} = 1.42, t_{Soon Lee, \diamond, 40^\circ C} = 0.13, t_{Soon Lee, \diamond, 45^\circ C} = 1.67, t_{Soon Lee, \square, 35^\circ C} = 0.30, t_{Soon Lee, \square, 40^\circ C} = 1.21, t_{Soon Lee, \square, 45^\circ C} = 0.76, P > 0.05$) between mated and unmated adults when exposed to temperatures of $\geq 35^\circ C$. The unmated adults, however, lived significantly longer ($df = 28, t_{KMelayu5, \diamond, 20^\circ C} = 5.60, t_{KMelayu5, \diamond, 27^\circ C} = 4.00, t_{KMelayu5, \square, 20^\circ C} = 11.11, t_{KMelayu5, \square, 27^\circ C} = 8.38, t_{Soon Lee, \diamond, 20^\circ C} = 7.20, t_{Soon Lee, \diamond, 27^\circ C} = 9.01, t_{Soon Lee, \square, 20^\circ C} = 9.94, t_{Soon Lee, \square, 27^\circ C} = 9.85, P < 0.05$) at 20°C and 27°C. These differences in the length of survivorship may be

because of repeated mating, which reduces the adult's longevity (male and female), as previously reported by Omori (1941). The reduced longevity of mated males in our studies is likely to be consequence of abdominal wounds from bed bug homosexual interactions, which may lead to higher desiccation rate (Ryne 2009). Also reported in other insects, the energy cost involved during mating process could also be another contributing factor for reduced longevity in mated males (Cordts and Partridge 1996, Kotiaho and

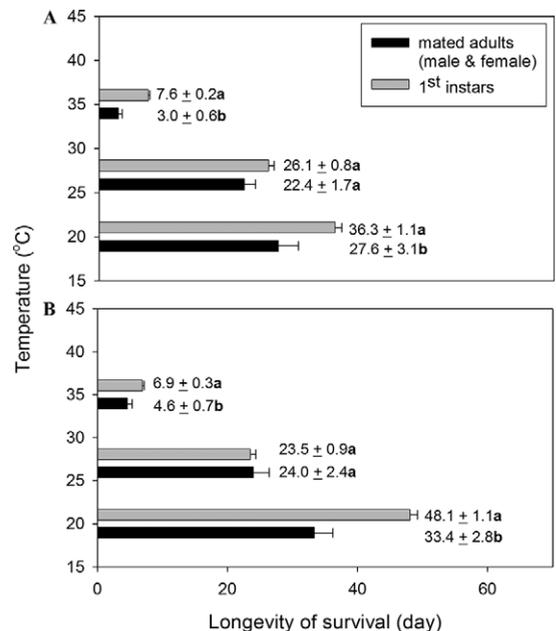


Fig. 2. Comparison of survival between first instars and mated adult *C. hemipterus* when exposed to various temperatures. (A) KMelayu5; (B) Soon Lee. Different letters within the same temperature indicate significant differences ($P < 0.05, t$ test) between first instars and mated adults.

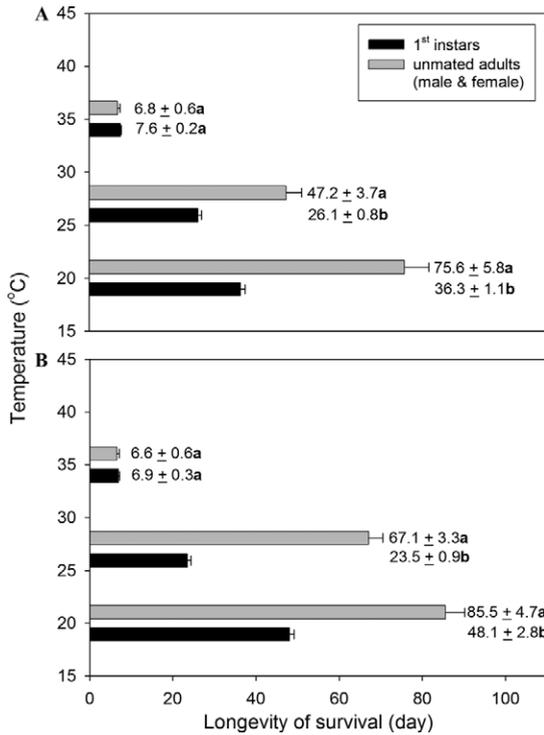


Fig. 3. Comparison of survival between first instars and unmated adult *C. hemipterus* when exposed to various temperatures. (A) KMelayu5; (B) Soon Lee. Different letters within the same temperature indicate significant differences ($P < 0.05$, t test) between first instars and unmated adults.

Simmons 2003, Burton-Chellew et al. 2007, Simmons and Kotiaho 2007). However, this observation warrants further investigation before the above proposition can be substantiated. The results prompted us to use unmated adults for the following survival and water loss studies instead of mated adults.

Omori's (1941) biological study of *C. hemipterus* and *C. lectularius* from Taiwan included data on the survival rate over a random combination of temperatures and RHs. The survival rate of first instars and adults was much longer in our study, whereas the egg incubation period was relatively shorter when compared with those reported by Omori (1941). In a study by Omori (1941), the egg incubation period was as long as 17.1 d at 20°C under 83% RH, which was far longer (by 5 d) than our longest egg incubation period at same temperature and 75% RH. The first instars in our study survived almost twice as long as those in the study by Omori (1941) at room temperature (27°C and 75% RH). In addition, unmated male and female adult *C. hemipterus* in our study had a maximum survival time that was 30–40 d longer than that in the study by Omori (1941) at room temperature. These variations likely are the result of differences in strain, food, and rearing conditions of the studies.

The univariate test ($r^2 = 0.925$) on unmated adults indicated that bed bug's survival was significantly ($P < 0.01$) influenced by the four main factors, namely

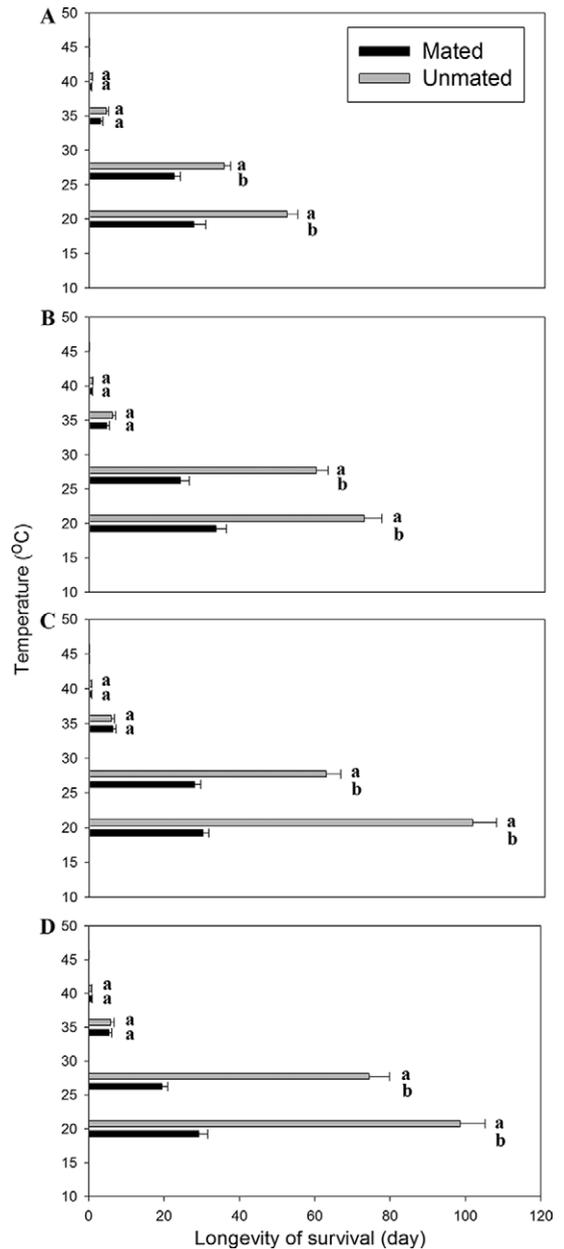


Fig. 4. Comparison of survival between mated and unmated adult *C. hemipterus* when exposed to various temperatures. (A) KMelayu5 males; (B) KMelayu5 females; (C) Soon Lee males; (D) Soon Lee females. Different letters within the same temperature indicate significant differences ($P < 0.05$, t test) between mated and unmated adult males or females.

temperature ($df = 4$, $F = 1500.77$), RH ($df = 2$, $F = 717.72$), strain ($df = 1$, $F = 80.06$), and sex ($df = 1$, $F = 77.42$). The effects of all interactions among these four factors were found to be significant ($P < 0.01$) in bed bug survival, excluding two interacting factors, as follows: strain and sex ($df = 1$, $F = 5.09$), and temper-

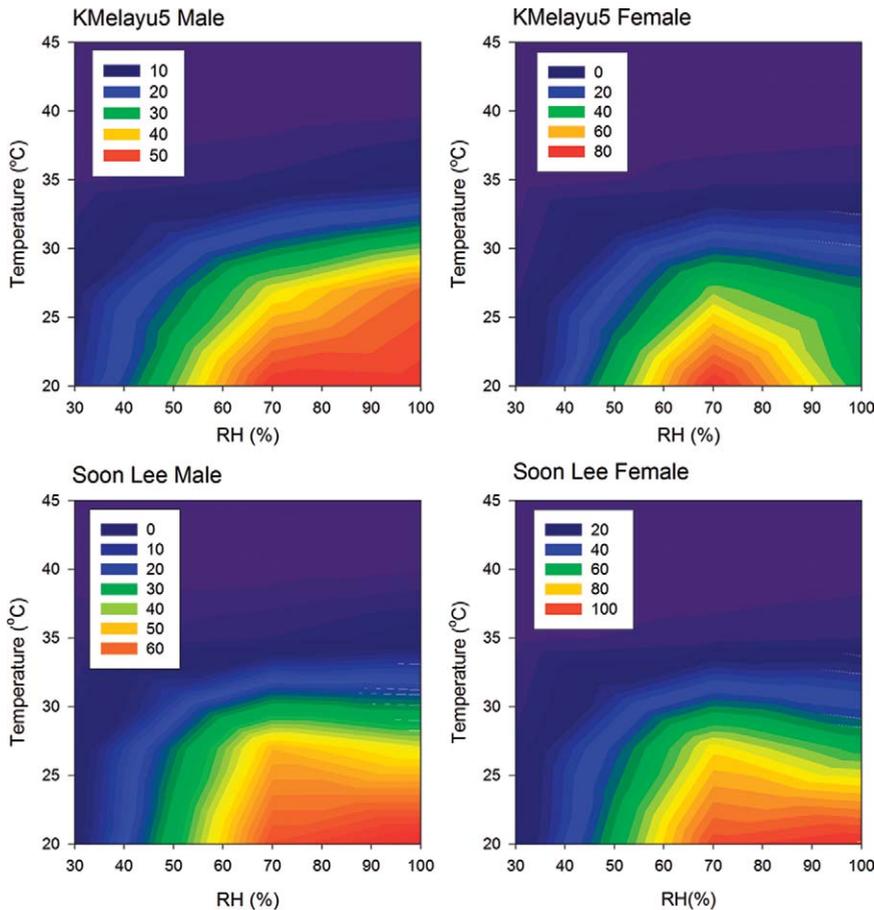


Fig. 5. Relationship between temperature and RH on the survival time of adult *C. hemipterus*. Different colors denote the survival time of the bed bug (in days). (Online figure in color.)

ature, strain, and sex ($df = 4$, $F = 3.02$). Stepwise regression revealed that the effects of both strain and sex on survival of bed bugs were significant ($P < 0.01$), but only with minimal influence. Post hoc comparison of temperature and RH further showed that bed bug survival was reduced significantly (Tukey's honestly significant difference test, $P < 0.01$) with increasing temperature and at lower humidity (33% RH), compared with that at higher humidity (75 and 100% RH).

Generally, the effect of temperature overrode the effect of humidity. When temperature is high, the effect of the humidity on the survival of bed bugs is almost negated. No significant difference was found between the survival time of adult males and females at the higher temperature ($\geq 35^\circ\text{C}$) and low humidity (33% RH) regime (Fig. 5). *C. hemipterus* had the highest survival time at the optimum condition of 20–25°C and 50–100% RH (Fig. 5). Males and females could survive a mean of 50–60 d and 80–100 d, respectively, after the last blood meal, which was given before the initiation of the experiment. This was obvious that bed bugs survived longer across the gradual decrease of RH, in which significant survival time reduction was observed at $\leq 33\%$ RH. Benoit et al.

(2007, 2009) also stated that the survival of *C. lectularius* declined significantly at $\leq 33\%$ RH. The authors also found that bed bugs were well adapted at the range of 50–75% RH.

Omori (1941) stated that high moisture conditions were detrimental to the survival of adult *C. lectularius* and partially to adult *C. hemipterus*. Our results also showed that high moisture conditions could shorten the survival rate of adult *C. hemipterus*, as observed at 20°C and 27°C. The lower survival time of bed bugs at these temperatures and humidity (100%) could be the result of the long-term exposure and intake of saturated water vapor, as all stages of bed bugs possess a critical equilibrium activity of $\geq 0.99a_v$ ($a_v = \%RH/100$) (Benoit et al. 2007). Other insects, such as *Haemagogus* mosquitoes, exhibited shortened survival rates when exposed to extremely high humidity (Bates 1947).

Water Loss in Relation to Temperature and Humidity. The univariate test ($r^2 = 0.912$) indicated that three factors, temperature ($df = 4$, $F = 425.93$), exposure time ($df = 5$, $F = 615.25$), and RH ($df = 2$, $F = 223.64$), had significant ($P < 0.01$) effects on the water loss of *C. hemipterus*. Significant interactions ($P <$

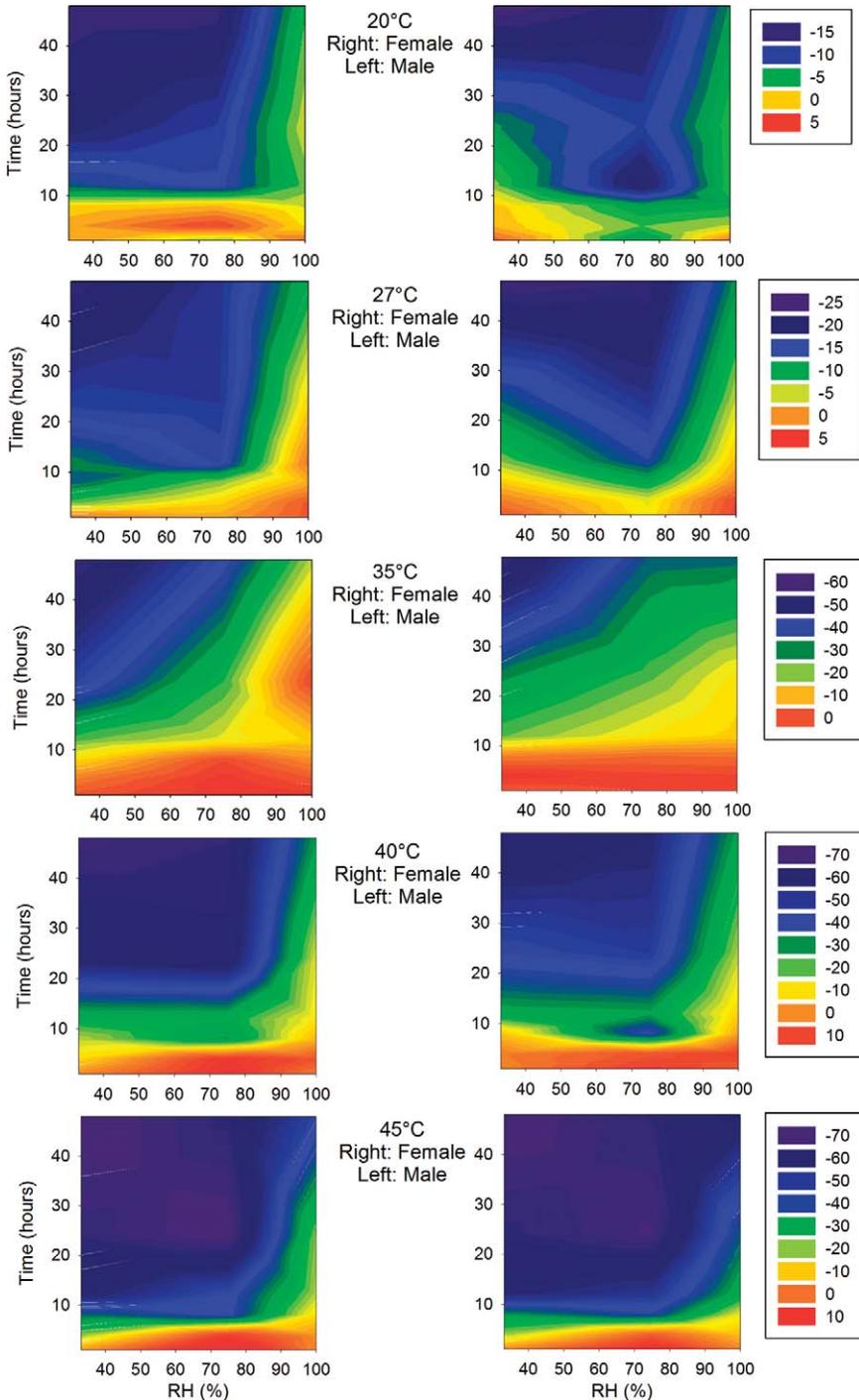


Fig. 6. Effects of the RH and exposure time on the water loss in adult *C. hemipterus* at various temperatures. Different colors denote the water loss of the bed bug (in %). (Online figure in color.)

0.01) among these three factors also were detected. No significant ($P > 0.05$) effect of sex ($df = 1, F = 0.24$) and strain ($df = 1, F = 2.56$) on water loss in *C. hemipterus* was detected, whereas three interactions, temperature and strain ($df = 4, F = 6.81$), RH and sex

($df = 2, F = 11.56$), and exposure time, RH, and sex ($df = 10, F = 2.81$), showed significant ($P < 0.01$) effects. Figure 6 exhibited the interaction effects of RH and sex that males were more susceptible to water loss than the females, particularly at higher tempera-

ture (35°C and 40°C) or at certain humidity (particularly at 33% RH). For example, males experienced $\geq 40\%$ water loss after 15 h, whereas females only encountered the same condition after 25 h, when both were exposed to 35°C at 33% RH. This was well supported by a general theory that water loss is positively correlated with total surface area and smaller body size (because male has smaller body size when compared with the female) (Edney 1957), in our case, applied in relation to interaction between exposure time, RH, and sex.

Overall, the test insects did not experience high water loss when exposed to 20°C and 27°C at three different RHs; under these conditions, water losses registered between 0 and 25% (Fig. 6). Bed bugs possess xerophilic characteristics, which are beneficial traits for water conservation, and a high critical transition temperature ($>35^\circ\text{C}$). These are similar to the characteristics of desert-adapted arthropods, which are able to tolerate a loss of one-third of their body water (Benoit et al. 2007). At 35°C and 33% RH, adult bed bugs experienced water loss of $\approx 40\text{--}60\%$ after 48 h (Fig. 6), and they only survived a mean of 1.50–3.20 d. Figure 6 showed that at 40°C, 75% RH, and after exposure of 15–20 h (mean survival time of males and females), the tolerable water loss range was between 35 and 45%.

This observation also was made in other studies (Kemper 1936, Edney 1957) that stated that cuticular permeability and water loss increased exponentially across increasing temperatures. At high temperature of 45°C, all bed bugs died after 2 h (irrespective of RH levels), although only 10% water loss was recorded (Fig. 6). This indicated that water loss is not the sole contributing factor to bed bug mortality at high temperature ($\geq 45^\circ\text{C}$), but other possible factors may also be involved, such as protein denaturation and imbalance of metabolic processes (Chapman 1998).

In this study, the bed bugs were treated in clusters during experiment. Because of the behavior of bed bugs in cluster, they were better able to conserve water than when individually kept. Benoit et al. (2007, 2009) reported earlier that there is a positive relationship between aggregation sizes and desiccation tolerance in *C. lectularius*. Similar observation has also been reported on other insects, including cockroaches (Yoder and Grojean 1997, Dambach and Goehlen 1999), beetles (Wolda and Denlinger 1984, Yoder et al. 1992), and cave cricket (Yoder et al. 2002).

Summary and Conclusion. We found that the upper thermal lethal limit for first instars and adults of *C. hemipterus* was 44°C and 46°C, respectively. The optimal temperature of egg hatchability was 35°C (with a shortened incubation period of <3 d and complete hatchability), and the eggs died completely at $\geq 39^\circ\text{C}$. Bed bug survived longer with increased RH, but extremely high RH (100%) might affect the survival rate of *C. hemipterus*. In general, the effect of RH decreased with increasing temperature, in which the adults showed the highest survival time at 20–25°C and 50–100% RH. *C. hemipterus*'s survival was significantly affected by temperature, RH, strain, and sex (either

singly or in interaction), but the effects of strain-sex in interaction and temperature-strain-sex in interaction were not significant. Water loss in *C. hemipterus* was significantly affected by temperature, exposure time, and RH (either singly, or in interaction).

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