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THERMAL TOLERANCE OF THE BED BUG

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Abstract Exploitation of very high or low temperatures for bed bug**,** *Cimex lectularius***,** control can be a supporting measure or even an alternative to chemical control methods. To observe the influence of heat, groups of adults, juveniles and eggs (group size = 20 individuals) were exposed to ambient temperatures T_A of 43°C and 44.6°C at exposure times of 10 to 40 min. After heat exposure, the adults and juveniles were kept at 25°C and eggs at 32°C. Bug activity was controlled following heat exposure after 7 d. All bed bugs (adults, juveniles and eggs) were dead after an exposure time of 30 min at T_A 44.6 °C. At this T_A, adults were less heat resistant (97.5 % survivors after 15 min heat exposure, no survivors after 20 min exposure) compared to eggs (still 17.5 % hatches after 25 min heat exposure, no hatches after 30 min exposure). To investigate the effect of cold, adult bed bugs were fed and then kept for 2, 6, 7, 8, and 9 days at a temperature of 25°C and 45 % RH. before they were transferred to 4.5°C and 55 % RH. (200 bed bugs per experiment). Bed bugs which were kept for 6 days at 25°C before they were exposed to 4.5°C had the highest survival rates (50% survivors after 99 d, n = 200). Bed bugs which were exposed to cold 2 or 9 days after their last blood meal had lower survival rates (50% survivors after 36 d and 69 d, respectively). Bed bugs stored at T_A 16°C had survived longer than at 4.5°C (50% survivors after 157 d and 99 d, respectively). Data from our experiments could be useful for a better understanding of heat or cold treatment of bed bugs.
Key words *Cimex lectularius*, lethal thermal exposure time, heat, cold, survival, metabolic rates, body mass

INTRODUCTION

Bed bug (*Cimex lectularius*) infestations have become an increasing public health problem during the last decade (Harlan et al. 2008, Reinhardt and Siva-Jothy 2006). Although bed bugs are no disease vectors, their bites cause cutaneous and systemic reactions (Goddard and deShazo, 2009). Bed bugs hide in small crevices and small populations are not easy to detect. Control of bed bug infestations may be challenging due to insecticide resistance and health concerns about spraying contact insecticides in living quarters. Hence, exploitation of very high or low temperatures for bed bug control can be a supporting measure or even an alternative to chemical control methods (Pinto et al., 2007). Upper lethal temperature for *Cimex lectularius* depends on exposure time and can be as low as 43°C when exposure is 100 min (Pereira et al., 2009). Initial knock down due to heat exposure must not be confused with mortality, since bed bugs can recover, although long-term survival rates of bed bugs treated with sublethal temperatures have not been determined (Pinto et al., 2007). In principle cold can be as lethal for insects as heat.

Bed bugs cannot tolerate freeze, and are killed when exposed to -16°C for 60 min (Benoit et al., 2009). However, up to now survival rates have not been determined for cold temperatures above the freezing point. In our study, we investigate both the effects of heat as well as cold temperature above the freezing point on the survival of bed bugs. For this end, we exposed bed bugs to heat within the known lethal range and observed the long-term survival of bed bugs at low temperatures with varying nutritional status before exposed to cold. To understand the effects of feeding on thermotolerance, we also measured metabolic rates of bed bugs directly and some days after their last blood meal.

MATERIAL AND METHODS

Bed bugs

All bed bugs used in the experiments were from a rabbit-adapted laboratory colony kept at the Federal Environment Agency. The FEA bed bug strain has the following characteristics: The bed bugs are fed weekly on chinchilla-bastard rabbits, the time needed for blood uptake is 10 to 15 min. The bed bugs are kept on filter paper (Ø 7 cm) in Petri dishes at an ambient temperature (T_A) of 25±2°C and a relative humidity (RH) of 45±10%. Bed bug eggs are collected each week and kept in separate Petri dishes at T_A 32±2°C and RH of 45±10%. Juveniles (J_1) hatch after 7 days, and their first ecdysis can be observed after 5 to 6 days. After 5 moultings in 5 to 7 weeks, the bed bugs develop into adults with a sex ratio of 1:1. Females lay about 10 eggs per week up to a biological age of about 110 days.

Experiments on Survival at 4.5°C

We investigated the influence of the transfer time for warm to cold temperatures after the last blood meal. For this end, adults which had received 7 to 8 weekly blood meals and juveniles (J_4, J_5) which had received 5 weekly blood meals during their development were kept in Petri dishes on filter paper at T_A 25±2°C and RH 45±10%. After their last blood meal, bed bugs were kept for a transfer time of 2, 6, 7, 8, and 9 days at T_A 25±2°C and RH 45±10% without further feeding, and were then transferred to a refrigerator with a T_A of 4.5°C and RH 55%. For a transfer time of 2 days at T_A 25±2°C and RH 45±10% before cold exposure, 3 experiments with 10 Petri dishes with 20 bed bugs (10♂/10♀) each was conducted as well as 1 experiment with 10 Petri dishes and 20 juvenile bed bugs (J_4, J_5; sex ratio not determined). For transfer times of 6, 7, 8, and 9 days at T_A 25±2°C and RH 45±10% before cold exposure, 1 experiment with 10 Petri dishes with 20 bed bugs (10♂/10♀) per each transfer time was conducted. The Petri dishes were inspected at least once a week and mortality rates were determined. Lethal thermal exposure time was calculated by transformation of time with natural logarithm and transformation of survival data after Weibull. Lethal thermal exposure time were 50% of the bed bug population were dead ($LTET_{50}$) was then calculated with linear regression.

Experiments on Survival at 16°C

Adult bed bugs which had received 8 weekly blood meals during their development were kept in Petri dishes at T_A 25±2°C and RH 45±10%. After their last blood meal, the bed bugs were kept for another 6 days at the same climatic conditions without further feeding before they were transferred to a chilling incubator with a T_A 16°C and RH 55%. The Petri dishes were inspected weekly and mortality rates were determined.

Influence of Heat Exposure on Survival

Bed bug adults (10♂/10♀) and juveniles (J_4, J_5) in this experiment received 6 to 7 blood meals during their development (i.e. 1 to 2 blood meals as adults) kept in Petri dishes at T_A 25±2°C and RH 45±10%. After their last blood meal, the bed bugs were kept for another 6 days at the same climatic conditions before they were exposed to heat.

Two different heat treatments were investigated: i) Adult bed bugs were exposed to 43°C in pre-heated glass test tubes (Ø 18 mm) in a laboratory water bath at different exposure times. T_A was controlled in the test tubes. Number of bed bugs per test tube was 10, and 4 to 6 test tubes were investigated per trial with 6 trials for each exposure time. Exposure times at T_A 43°C were 25 min (n = 290 bugs), 30 min (n = 350), 35 min (n = 350), 40 min (n = 260) and control (no heat exposure, n = 100 bugs). After heat exposure, bed bugs were transferred to Petri dishes (25±2°C and RH 45±10%) and observed for mortality 1h, 12h, 48h, 72h, and 6d after heat exposure; ii) To simulate the situation in a bed bug hideout (thermal isolation) during heat treatments, bed bugs or bug eggs were placed in a wooden tube (L 50 mm, wall thickness 2 mm, Ø 11 mm) inserted in glass test tubes in a laboratory water bath with a constant temperature of 45°C. In addition to juveniles and adults, we also investigated the effect of heat exposure on eggs. Eggs were collected from the laboratory colonies and had an age of 1 to 5 days when exposed to heat. After the bugs were inserted to the wooden tube, it was placed in the water bath and the temperature was measured directly in the wooden tube. Bed bugs were exposed for 10, 15, 20, 25, and 30 min to heat. 20 bugs (10♂/10♀) were inserted per wooden tube, with 2 test tubes per trial and 2 trials for each exposure time. After heat exposure, bed bugs and eggs were transferred to petri dishes (adults and juveniles 25±2°C and RH 45±10%, eggs 32±2°C and RH of 45±10%) and observed for mortality 1h, 6h, 24h, 48h, and 72h, after heat exposure of adults and juveniles. Eggs were observed 7 d after heat exposure and hatch rates were recorded.

Metabolic Rates

7 days after their last blood meal, the body mass of 10 individual adult female bed bugs was determined with an electronic fine balance (Bp 61S, Sartorius, Göttingen, Germany). Subsequently, the bugs received a new blood meal and their body mass was weighted again, and then daily for 13 days.

Calorimetric experiments were performed to determine the metabolic rate (i.e. heat production rate) of *Cimex lectularius* at different T_A. For this end, an isoperibolic batch microcalorimeter (Biocalorimeter B.C.P.; Electronique Arion, Orsay, France) with sensitivity of 44.3 µV/mW was used. The calorimeter contained a measuring and a reference vessel with volumes of 12 ml each and was connected to a chart recorder (BD 111900750; Kipp and Zonen, Delft, The Netherlands). The metabolic rates of adult bed bugs were determined with groups of 20 bed bugs 1 day and 9 days after last blood meal, respectively. Experiments were performed at T_A 30°C (n = 13), 35°C (n = 10), 40°C, (n = 5), 42 °C (n = 13), and 43°C (n = 5). The bugs were weighed and transferred to the calorimeter measuring vessel, and their heat production rate was recorded for 90 min.

In addition to experiments on metabolic response against different T_A, the heat production rates of groups of 20 bed bugs each with different nutritional status were determined. Heat production rates of bed bugs 1 day after their last blood meal (n = 13), 5 days (n = 4), 6 days (n = 4), and 9 days after their last blood meal (n = 8) were measured at T_A 30°C for 90 min.

RESULTS

Experiments on Survival at 4.5°C

Survival rate of bed bugs was dependent on the time between last feeding and the transfer to cold temperature (Figure 1). Highest survival rates were observed when bed bugs were kept for 6 days after their last blood meal at 25°C before they were exposed to 4.5°C. The lethal thermal exposure time were 50% of the bugs were killed ($LTET_{50}$) was 99 d, and ≥95% were killed after 197 d. $LTET_{50}$ was shortest when the bed bugs were exposed to cold 2 days after their last blood meal and amounted to 36 d (≥95% after 67 d). Juveniles (J_4, J_5) with this treatment were even less cold resistant; $LTET_{50}$ was only 20 d (≥95% after 57 d). Bed bugs with a transfer time of 7, 8 and 9 d at 25 °C before cold exposure had $LTET_{50}$ of 62, 79 and 69 d; (≥ 95% were killed after 150 d, 163 d and 146 d, respectively).

Experiments on Survival at 16°C

The lethal thermal exposure time for incubation at 16°C were 50% of the bugs were killed ($LTET_{50}$) was 157 d. After 90 d, near the $LTET_{50}$ at 4.5°C for bed bugs with the same transfer time between last blood meal and transfer to cold, 90% of the bed bugs were still alive.

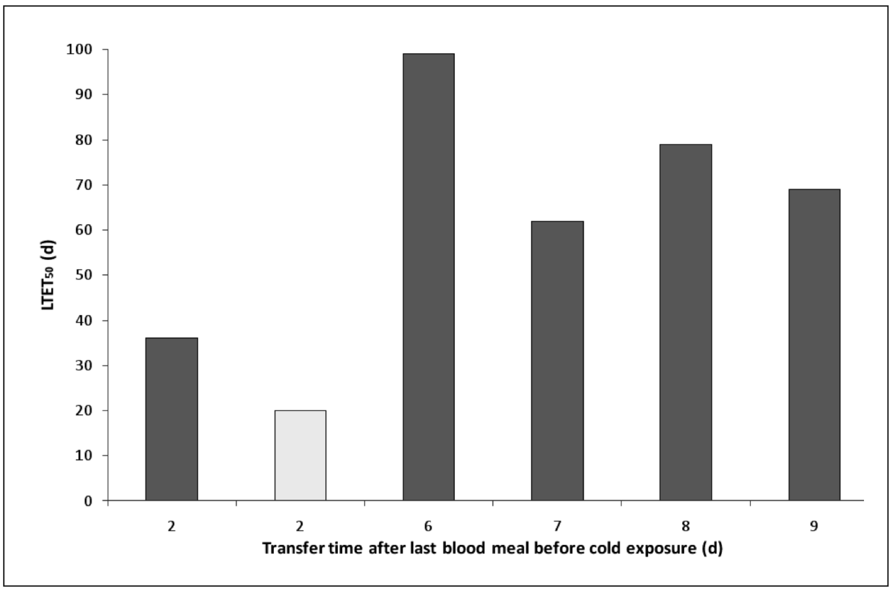

Figure 1. Influence of nutritional status of bed bugs on survival before cold exposure at 4.5°C. Bars indicate the lethal thermal exposure time were 50 % were killed ($LTET_{50}$). Light grey bar indicates data for juveniles (J_4, J_5), dark bars indicate data for adults. Transfer time 2 d (adults) n = 600 bed bugs; 2 d (juveniles) n = 200; 6 d, 7 d, 8d, and 9 d n = 200 each.

Influence of Heat Exposure on Survival

At T_A 43°C, bed bug survival was dependent on heat exposure time. After exposure of 25 min, 94% of the treated bed bugs were still alive 6 d after exposure (Table 1), whereas 40 min of heat exposure at 43°C killed nearly all bed bugs.

Table 1. Survival rates of adult bed bugs at different heat exposure times at T_A 43 °C. SD = standard deviation.

Heat exposure time (min)	**25**	**30**	**35**	**40**	**Control**
Survival rate (± SD)	94 ± 0.6	71.2 ± 0.5	24.2 ± 1	0.75 ± 1	100
n	290	350	350	260	100

Table 2. Heat exposure of bed bugs in a simulated harborage. Table head shows temperature development during heat exposure. Table body shows survival and hatch rates of bed bug adults, juveniles and eggs under this temperature conditions. n.d. = not determined

Time (min)	0	4	8	10	12	20	25	30
Temperature (°C)	21	30	40.5	44	44.5	44.6	44.6	44.6

Development stage	**Adults**		**Juveniles (J_3)**		**Eggs**
Heat exposure time (min)	Survival rate (%) n = 80 for each exposure time		Survival rate (%) n = 80 for each exposure time		Hatch rate (%) n = 80 for each exposure time
	after 24 h	after 7 d	after 24 h	after 7 d	after 7 d
10	97.5	94	100	74	88.4
15	97.5	81	99	52	69
20	0	0	97.5	0	33.8
25	n.d.	n.d.	0	0	17.5
30	n.d.	n.d.	n.d.	n.d.	0
Control	98.7	98.7	100	100	90

Influence of Heat Exposure Under Simulated Habitat Conditions

Table 2 shows the temperature increase in the wooden test tubes after the bugs were inserted and the tubes were transferred into a water bath. Adult bed bugs were most heat sensitive and needed shorter heat exposure times than juveniles and eggs. Eggs were the most thermotolerant life stage, and 100% mortality (i.e. no hatches) could be observed after 30 min.

Metabolic Rates

The body mass of female bed bugs 7 d after their last blood meal amounted to 3.49 mg (SD ± 0.65 mg, n = 10). Immediately after their next blood meal, their body mass was 14.5 mg (SD ± 2.1 mg), the weight gain was thus in average 315 %. Within the following 10 days, the females lost in average 9.6 mg or about 66% of their body mass. Figure 2 shows the average body mass decrease after a blood meal together with the accompanying decrease in heat production rate from 2.1 mW/g (n = 13) at day 1 to 0.9 mW/g (n = 8) at day 9.

Heat production rates of bed bugs did increase only slightly with increasing T_A between 30°C and 40°C, and then increased drastically at T_A 42°C and 43°C (Figure 3).

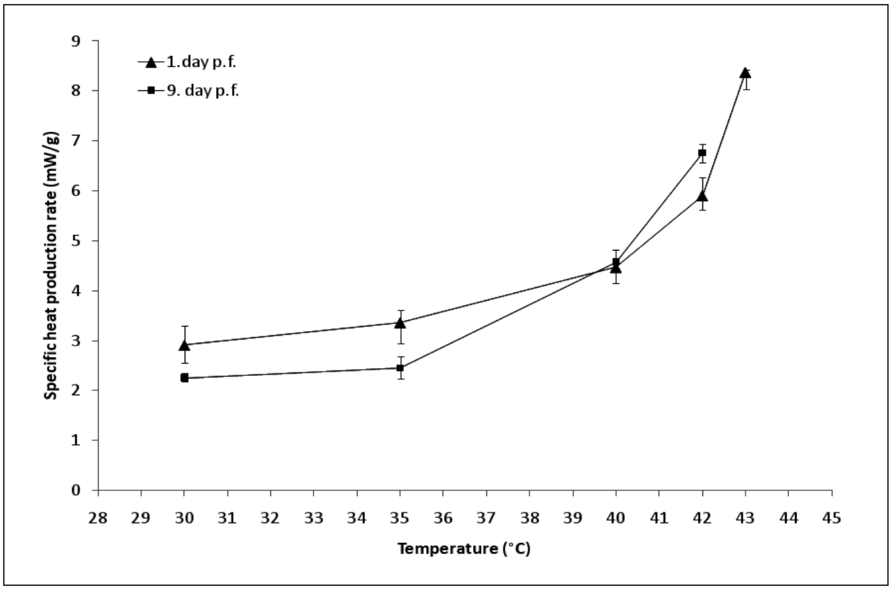

Figure 2. Calorimetrically determined heat production rates of bed bugs as function of ambient temperature. Symbols denote the median, upper bars denote 3. quartile, lower bar 1. quartile, triangles: bed bugs 1 day after their last blood meal (T_A 30°C n = 13, 35°C n = 10, 40°C n = 5, 42°C n = 13, 43°C n = 5) , squares: bed bugs 9 days after their last blood meal (T_A 30°C n = 8, 35°C n = 4, 40°C n = 4, 42°C n = 5)

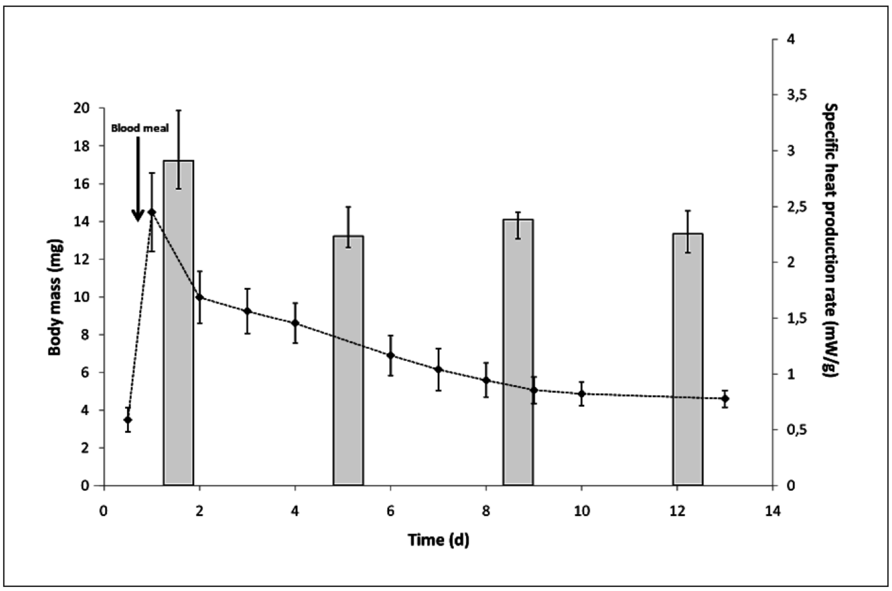

Figure 3. Body mass change and heat production rates of bed bugs with different feeding status. Squares connected with dotted line denote mean body mass (n = 10 per data point), error bars indicate standard deviation; grey bars denote median for specific heat production rate, error bars indicate 1. and 3. quartile, day 1 after last blood meal n = 13, day 5 n = 4, day 6 n = 4, day 9 n = 8.

DISCUSSION

Thermal exposure to T_A 43°C for 40 min killed nearly all adult bed bugs, confirming date from Pereira et al (2009). Data from other sources that exposure to T_A 40°C for more than 15 min is lethal (Cooper and Harlan 2004) could not be confirmed, since the survival rate at T_A 43°C after an exposure time of 25 min was 94 % (observation after 6 d), although the survival rate decreased drastically with longer exposure times. Eggs were the most heat resistant life stage, which is in accordance with the information given by Cooper and Harlan (2004). Following an exposure of 44.6°C for more than 20 min (Table 2), 17.5 % of the exposed eggs still produced hatchlings after one week. Interestingly, juveniles seem to be more heat tolerant than adults are, and an heat exposure of 15 min in our experiment with simulated habitat conditions with rising T_A from 21°C to 44.6°C, 99 % survived the first 24 h after heat exposure and about 50% were dead after one week. Bed bugs show a rapid increase in metabolic rates when exposed to $T_A \geq 42°C$ (Figure 3), which is an indication for heat stress.

Bed bugs are vulnerable to subzero temperatures, but they are able to survive $T_A > 0°C$ (Benoit et al., 2009; Quarles, 2007). Longevity is higher at T_A 10°C were the average survival of adults is 413 d, compared to T_A 27°C with a survival of only 65 d (Quarles, 2007). In our experiments, bed bugs with the same nutritional status survived longer at T_A 16°C ($LTET_{50}$ 157 d) than at T_A 4.5°C ($LTET_{50}$ 99 d), indicating that moderately cold conditions with T_A between 10°C and 16°C may enhance survival, whereas T_A above or below these values decrease longevity. Bed bugs increase their weight considerably after feeding, and the digestion of the blood meal can be followed by measuring body mass loss. 24 h after feeding, the weight loss is most pronounced and metabolic rates are highest. Within the following 10 to 12 d, bed bugs lose body mass constantly whereas the metabolic rates stay on the same level (Figure 1). From day 9 to day 13 after blood meal, the weight loss is only about 0.2 to 0.3 mg/d, and Hase (1930) presents data that the whole blood meal is completely digested within 15 d at T_A 18 to 20°C. Interestingly, the survival at cold temperature is best when the bed bugs were given 6 d to digest their last feeding ($LTET_{50}$ 99 d). When they had only 48 h for processing their blood meal survival rates were much lower ($LTET_{50}$ 36 d). Our results indicate that nutritional status and feeding history have to be taken into account when the thermal biology of bed bugs is investigated.

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