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Rapid killing of bed bugs (*Cimex lectularius* L.) on surfaces using heat: application to luggage

Catherine Loudon*

Abstract

BACKGROUND: The resistance of bed bugs (*Cimex lectularius* L.) to chemical insecticides has motivated the development of non-chemical control methods such as heat treatment. However, because bed bugs tend to hide in cracks or crevices, their behavior incidentally generates a thermally insulated microenvironment for themselves. Bed bugs located on the outer surface of luggage are less insulated and potentially more vulnerable to brief heat treatment.

RESULTS: Soft-sided suitcases with adult male bed bugs on the outside were exposed to an air temperature of 70–75 °C. It took 6 min to kill all of the bed bugs, even those that had concealed themselves under zipper flaps or decorative piping. During heating, only one bed bug (out of 250 in total) moved into the luggage (through a closed zipper). Over long periods of time (24 h) at room temperature, adult male bed bugs on the exterior of luggage only infrequently moved inside; only 3% (5/170) had moved inside during 24 h.

CONCLUSIONS: Brief exterior heat treatment of luggage is a promising way to reduce the spread of bed bugs being transported on the outer surface of luggage. This treatment will not kill bed bugs inside the luggage, but could be a component of integrated management for this pest.

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Keywords: bed bug; heat treatment; travel; control; *Cimex lectularius*

1 INTRODUCTION

Bed bugs (*Cimex lecturalius* L.) continue to spread in communities across the globe.[1–5] A variety of methods for control or detection have been described or are under development, but no single method has emerged as an inexpensive and effective control treatment that has displaced all others (reviewed in Cooper[6] and Koganemaru and Miller[7]). For example, the evolution of resistance to chemical insecticides in bed bugs has generated challenges for chemical control methods.[8–10] A combination of different tactics is emerging as an overall strategy to control and monitor infestations.[11] Non-chemical methods are generally considered to be an essential part of integrated management,[6,12] and strategies in use or in development include physical interceptor devices,[13,14] encasement for mattresses,[15] heating,[16] cooling,[17] biomimetic entrapment surfaces[18] and vacuuming.[1,7,12,19]

Heat treatment to kill bed bugs is one approach that avoids problems associated with chemical treatments. Decades ago, 45 °C was identified as an operative lethal temperature for bed bugs.[20,21] More recently, this use of a threshold lethal temperature has been updated by a recognition of the relationship between temperature and the exposure time required to cause 100% mortality in bed bugs. For example, 100% mortality of bed bugs required 100 min of exposure at 41 °C, but only 1 min of exposure at 49 °C (for bed bugs suddenly exposed to an elevated temperature).[16] While this would seem to suggest that heat treatment could be a rapid process, in practice it is not, because of the behavior and location of the bed bugs. Bed bugs tend to hide in cracks or crevices, and therefore become thermally insulated from an external source of heat. Long treatment times are necessary to heat the containing structures until the temperature at the location of the hidden bed bugs has risen to lethal levels, and bed bugs that are being slowly warmed may take longer to kill than bed bugs abruptly exposed to elevated temperatures.[22] It can take many hours to kill all of the bed bugs in single rooms or small houses.[22,23] Localized heat treatment of ensheathed furniture is faster, but still requires 2–7 h to bring the temperature sufficiently high at the deeper, insulated locations within the furniture.[16] In response to an increasing temperature, bed bugs can retreat further into an item that is being heat treated, or even spread an infestation if not contained.[11] A large item being heated will have a temperature gradient that may include cooler areas in which bed bugs can congregate and survive. For example, mattresses encased in black plastic and placed on the ground in full sun for 9 h reached a lethal temperature of >80 °C on the upper surfaces, but there were areas on the underside (next to the ground) that remained at sublethal temperatures (e.g. peaking at 36.5 °C) and therefore could serve as a refuge, leading the authors to reject this method as a reliable one for killing bed bugs.[24] Laundering is an effective way of using heat to kill bed bugs on clothing or linens: washing at 60 °C (90 min) or tumble drying at >40 °C (for >30 min) was sufficient to kill all stages of bed bugs, including eggs.[25] These shorter times are

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made possible by the agitation of the items in a washing machine or dryer, which speeds heat penetration as items are effectively heated from all sides.

Transport on or in luggage is one of the ways that bed bugs are spread.[26,27] Bed bugs located on the outside of luggage provide one of the few cases in which bed bugs are more constrained to the periphery of an object, and hence are less thermally insulated and potentially more vulnerable to heat treatment. A number of commercial portable heating chambers are currently available that reach temperatures of 50 °C or higher, and could be used for luggage as well as many other items. Hypothetically, a brief exposure to a relatively high temperature could kill the bed bugs on the outside without sufficient time to heat the interior of the luggage very much. This is reminiscent of steam treatment of the outside of furniture or mattresses, in which a brief exposure to steam kills all exposed bed bugs.[28] In order to evaluate the exposure time that would be required to kill bed bugs located on the outside of a piece of luggage with externally applied heat, bed bugs were placed on suitcases at room temperature, and the suitcases were transferred to a large heated chamber for varying time periods. Any tendency for bed bugs to move into luggage from the outside surface (crossing a zipper) was evaluated both while heating the outside of the luggage (up to a maximum of 6 min) and for unheated luggage (left at room temperature for 24 h). To extend our knowledge on the lethal limit of bed bugs for temperatures higher than those documented earlier (abrupt exposure up to 49 °C[16] and gradual increase up to 55 °C[22]), bed bugs were placed in preheated wells (45–70 °C), and the exposure time to produce 100% mortality was determined.

2 EXPERIMENTAL METHODS

2.1 Bed bugs

All bed bugs used for these measurements were adult males, and were obtained from Dr Kenneth Haynes (University of Kentucky, the 'Fort Dix' colony, collected >30 years ago; see Zhu *et al.*[9] for more details). Each bed bug was only used once. Bed bugs were maintained at UCI in an insect rearing room (20–22 °C) without feeding (after eclosion to an adult) in flat-bottomed glass vials (23 mm diameter) in test tube racks with a piece of paper in the bottom of each vial for harborage. The test tube racks were inside covered glass aquaria, placed on supports above a saturated solution (sodium chloride in water) that covered the bottom of the aquaria to a depth of a few centimeters. The solution acted as a moat and maintained the humidity in the aquaria at about 75%.[29] To avoid potential injury to the bed bugs associated with handling, bed bugs were never handled with forceps, and were either transferred by walking from one piece of paper onto another or by gently sliding from a tilted glass vial after walking off the paper. Immediately before use, bed bugs were transferred to vials without paper. To transfer a bed bug in a glass vial to luggage or a heated aluminum block, the glass vial was inverted, and the bed bug gently slid onto the luggage or into the well of a heated aluminum block.

2.2 Bed bugs exposed to heat in wells in an aluminum block

Individual bed bugs were placed in wells of a preheated aluminum block of a temperature-controlled 'dry bath' (GeneMate Mini Dry Bath, New Delhi, India) normally used to heat microcentrifuge tubes (each well is approximately 10 mm in internal diameter and 25 mm deep). The aluminum block was preheated to 45, 50, 55, 60, 65 or 70 °C (manufacturer's specifications for accuracy 0.5 °C). Temperatures were presented in a random order, changing after every ten bed bugs. Each bug was placed by itself into a single preheated well. Bugs were heated for time durations ranging between 1 and 120 s. Because handling an individual bug took about a second or two, only a single bed bug was measured at a time for shorter time durations (up to 60 s); up to three bed bugs were measured at a time (each in its own well) for longer time durations (>60 s). A minimum of four and a maximum of six different time durations were used for each temperature (the range of time durations was overlapping but slightly different for the different temperature treatments, with longer time durations used for cooler temperatures and shorter time durations used for warmer temperatures). When the set time duration was reached, the aluminum block was inverted and the bed bug(s) dropped onto a piece of paper at room temperature. 'Knockdown' was immediately assessed; if a bed bug showed any movement at all of any part of its body, it was not considered to be knocked down. After heating, each bed bug was placed in a single glass vial and stored at 20–22 °C until the next day. The day after heating, if a bed bug did not show any movement of any part of its body, even after gently touching it with a strip of paper, it was coded as dead. Ten bed bugs were used for each temperature/time duration combination, for a total of 310 bed bugs. None of the control bugs ($n = 30$; in test tubes, not heated) died during this 24 h period. Bugs used for these measurements were approximately 3 weeks after eclosion to adulthood.

2.3 Bed bugs exposed to heat on luggage

Up to five adult male bed bugs at a time were transferred to an exposed area on the outside of a piece of luggage, which was then placed inside a large preheated luggage conveyor chamber (Pur Systems, Lake Havasu City, AZ) set to 75 °C. Introduction of the luggage caused a small amount of cooling; the chamber temperature ranged between 70 and 75 °C during the measurements (mean of 73 °C, $n = 53$). The luggage was oriented with its long axis horizontal, and the bed bugs were placed on the upper surface, where most of the zippers were located. The chamber contained a vortex blower system to circulate the air gently, and was closed to the outside room air by vertical heavy-grade lead-lined strips. After a predetermined amount of time (ranging from 30 s to 6 min), the luggage was removed from the conveyor chamber and the bed bugs were recovered with their location noted (whether exposed on the surface, hidden in a crevice and therefore not visible without pushing the concealing piping or zipper to the side, or inside a zippered compartment). A total of 250 bed bugs were heated on luggage in this manner. Bugs ranged in age between 2.5 and 4 weeks post-eclosion (to adult stage). The temperature of the approximate middle of the upper surface of the luggage was measured using a thermal camera (model E30; FLIR Systems, Wilsonville, OR) (see Fig. 4B) within 5 s after removal from the conveyor chamber. The temperature of the inside of the luggage was tracked using an iButton (model DS1922L; Maxim Integrated, San Jose, CA) (see Fig. 4C) that had been placed inside. This iButton model, storing 1 reading s^{-1}, is accurate to 0.5 °C and has a thermal response time constant of 130 s. Only the maximum temperature for each heating event is reported. Luggage was allowed to cool for at least 18 min before reuse. For a control, bugs were placed on luggage in the same chamber in an identical manner to that described above but with the temperature set to match room temperature (24 °C) for 6 min (four sets of bugs for a total of $n = 20$ bugs).

2.4 Bed bugs left on unheated luggage

Bed bugs (all adult males) were placed on the outside of a piece of luggage and left for 24 h. Seventeen groups of ten bed bugs at a time were used, for a total of 170 bed bugs. The luggage was placed on a large plastic tray (70 × 75 cm), which was lined with paper to provide harborage if necessary for any bed bugs that left the luggage. Two large clear plastic bags (50 gal) completely enclosed the plastic tray and luggage to prevent escapees (double bagged). iButtons (DS1923 or DS1922L) were placed inside the luggage or outside the luggage (inside the enclosing plastic bags) to record the humidity (to nearest 0.6% RH) and temperature (to nearest 0.5 °C) every minute during the 24 h period. Averages for the 24 h periods are reported. Two light regimes were used: constant darkness or 16:8 light:dark. The light regime was alternated between successive measurements. After each 24 h period, the location of all bed bugs was noted. Bugs ranged in age between 1.5 and 8 weeks post-eclosion (to adult stage).

2.5 Luggage

Two different pieces of 'soft-sided' luggage were used: one suitcase was American Tourister (Brookfield line) and was 64 cm long by 45 cm across by 26 cm deep, and covered with blue fabric with zippers and piping, and the other suitcase was Samsonite, 41 cm long by 30 cm across by 18 cm deep, and covered with black fabric with zippers and piping. All zippers were closed during the measurements.

2.6 Statistics

Statistical tests were performed using SAS 9.4 (SAS Institute, Inc., Cary, NC). The Proc GLM (general linear models) procedure was used for regression, analysis of variation, analysis of covariance and tests for homogeneity of slopes. A test for homogeneity of slopes was performed prior to an analysis of covariance. Type III results are reported (in this model the order of the parameters does not matter, because each effect is adjusted for all other effects).[30] The Proc FREQ procedure was used for Fisher's exact test of the contingency table (Table 1). Fisher's exact test was used because the count in some of the cells was very low. Mortality data for the bed bugs placed in heated wells were evaluated using Proc PROBIT with the logistic option (as was done in Kells and Goblirsch[22]). The results from the logistic regression equations are reported as a function of y, from which the probability of mortality may be computed as $\Pr(\text{mortality}) = \phi(y)$, where ϕ is the normal cumulative distribution function.

The 95% confidence interval for a measured proportion $\hat{p}$ was calculated as

$$\hat{p} \pm 1.96\sqrt{\frac{\hat{p}\,(1-\hat{p})}{n}}$$

without using the formula adjustment method recommended for small sample sizes, because all group sample sizes for which these intervals were calculated were ≥18.[31–33]

3 RESULTS AND DISCUSSION

3.1 Lethal exposure times for preheated surfaces

Bed bugs suddenly exposed to a preheated surface (transferred to a well in a preheated aluminum block) died more rapidly with a higher temperature (Figs 1 and 2). The lower end of the experimental temperature range of the present study (45 and 50 °C) overlapped with the upper limit of the temperature range of previously published work (49 °C, Pereira *et al.*[16]), and the results were completely consistent with that earlier study. Exposure to 45 °C for 2 min did not kill any bed bugs (Fig. 1), which is consistent with Pereira *et al.*[16] At the slightly warmer temperature of 50 °C, 100% mortality was seen after 45 s of exposure (present study), again very similar to the 60 s exposure to 49 °C reported earlier (Pereira *et al.*[16]; 45 s exposure not used). Only a very brief 10 s of exposure was sufficient to kill all of the bed bugs at 65 °C or higher (Figs 1 and 2).

As noted by Pereira *et al.*,[16] there is an approximately linear relationship between the log-transformed minimum time needed for 100% mortality and temperature (in the range 41–50 °C) (Fig. 2); the present study found a slightly different log-linear relationship for the temperature range 50–65 °C. The difference in slopes between these two studies is unlikely to be the result of small differences in protocol or bed bugs because of the close agreement at the point of intersection of the two lines (Fig. 2). The changing relationship is more likely to reflect the different thermal situations corresponding to short times (<60 s) versus longer times (>60 s). When an object (bed bug, in this case) is heated externally, it takes time for the heat to penetrate into the object, and at shorter times only the periphery of the object will experience an increase in temperature. A dimensionless number, the Fourier number (*Fo*), is used in the physical sciences to characterize how far (characteristic length *L*) or for how long (time *t*) heat has penetrated an object of thermal diffusivity α:[34,35]

$$Fo = \frac{\alpha t}{L^2}$$

Heat conduction problems are often simplified whenever possible as $Fo >> 1$ (roughly enough time for heat penetration to depth *L*) or $Fo << 1$ (roughly insufficient time for heat penetration to depth *L*) because the solutions for these two cases are distinct and more simple than when $Fo \approx 1$.[36] The magnitude of a dimensionless number is useful for qualitative characterization when an exact solution such as an internal temperature profile is not necessary. Using the formula above, an order of magnitude estimate can be made for the time corresponding to $Fo \approx 1$, at which the gradual

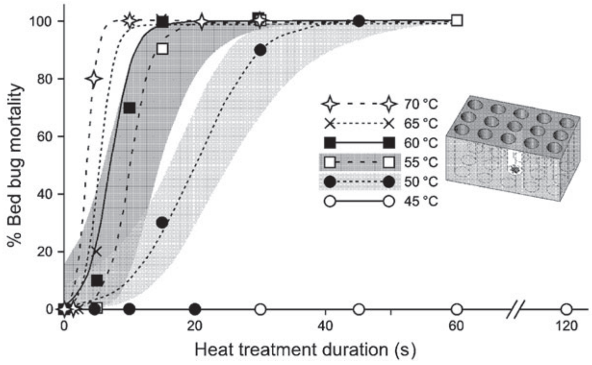

Figure 1. Mortality of adult male bed bugs after placement into preheated wells as a function of temperature and time of exposure. Logistic regression curves (dashed or solid lines) are provided for each temperature except 45 °C (there was no mortality within 2 min at 45 °C), with 95% confidence intervals provided for 50 and 55 °C (confidence intervals for temperatures ≥ 55 °C overlap extensively). Logistic regression results are: 50 °C $y = 8.85\ \log_{10}(\text{time}) - 11.38$, $\chi^2\ (1, n = 50) = 15.08$, $P < 0.0001$; 55 °C $y = 13.6\ \log_{10}(\text{time}) - 13.4$, $\chi^2\ (1, n = 40) = 6.67$, $P < 0.01$; 60 °C $y = 10.9\ \log_{10}(\text{time}) - 9.58$, $\chi^2\ (1, n = 40) = 6.07$, $P = 0.01$; 65 °C $y = 13.2\ \log_{10}(\text{time}) - 9.43$, $\chi^2\ (1, n = 50) = 5.38$, $P = 0.02$; 70 °C $y = 7.12\ \log_{10}(\text{time}) - 3.19$, $\chi^2\ (1, n = 60) = 10.54$, $P = 0.001$.

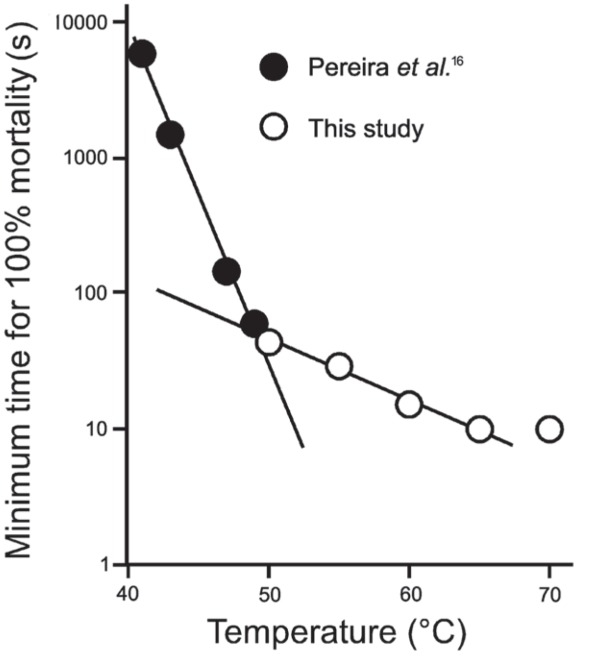

Figure 2. Minimum time for 100% mortality in adult male bed bugs follows a linear relationship between log(time) and temperature in the range 41–49 °C ($y = -0.25x + 13.98$, $r^2 = 0.996$; four points from Table 1 in Pereira *et al.*[16]) and a different linear relationship between log(time) and temperature in the range 50–65 °C ($y = -0.045x + 3.93$, $r^2 = 0.99$), where $y = \log10(\text{time, s})$ and $x =$ temperature (°C).

transition occurs between a bed bug being heated primarily at its surface ($Fo << 1$) and heated through its whole body ($Fo >> 1$). The thermal diffusivity of a bed bug is unknown, but has been reported for shrimp as $\alpha = 1.4 \times 10^{-7}$ m^2 s^{-1}.[37] This shrimp estimate for thermal diffusivity is no different from the thermal diffusivity of water,[34] suggesting that this number is a reasonable approximation for any arthropod, including bed bugs. Solving for time t, using $Fo \approx 1$, $L \approx 2$ mm (relevant heat-penetrating depth into the bed bug) and $\alpha = 1.4 \times 10^{-7}$ m^2 s^{-1}, results in an estimate of 30 s. This time of 30 s is the same order of magnitude as the time at the observed intersection between the two lines (Fig. 2), suggesting that different slopes of the two lines may correspond to different heating situations. From a pest control perspective, any exposure under a minute is very fast.

Bed bugs dropped into heated wells made vigorous, jumping movements for the first few seconds. These movements temporarily reduced contact between the bed bug and the solid heated surface, which presumably reduced the initial heating rate of the bed bugs. This behavior is probably what caused the ‘plateau’ of 10 s (Fig. 2) for temperatures of 65–70 °C.

Less time was required to achieve 100% knockdown (immobilization) at higher temperatures. A time of 60 s was necessary to achieve 100% knockdown at 45 °C, 15 s was necessary to achieve 100% knockdown at 50–55 °C or higher and only 10 s was necessary to achieve 100% knockdown at 60–70 °C. As reported earlier,[16] many of the knocked-down bed bugs recovered, at least to the extent that they were still alive the next day. Out of the total of 310 bed bugs, 236 were knocked down, and 36% of these knocked-down bed bugs recovered by the next day (86 out of the 236 knocked-down bed bugs). From a control perspective, the time at which bed bugs become immobilized is important because after this point they are no longer able to move to a safer area (and will in fact be killed if the temperature remains elevated for a sufficiently long period of time).

Mortality rates of bed bugs abruptly subjected to a fixed, high temperature lie at one end of a continuum of possible heating regimes, and thus provide a useful standard for comparison. Bed bugs subjected to heat treatment as a control method only rarely experience a sudden high temperature; this is most closely approximated for unconcealed bed bugs (e.g. exposed on the outside of furniture) hit with steam[28] or placed in a preheated chamber. Even under exposed conditions, the proximity and thermal inertia of the furniture or luggage will mitigate the rate of heating of the bed bug on the surface (a function of the thermal characteristics of the article[34]). Under usual heat treatment conditions, in which an entire room or large article is being treated, and the bed bugs are able to self-insulate by moving around, the bed bugs will experience a gradual ramping or increase in temperature at their location, which they can control to a certain extent by their movements to cooler locations. Kells and Goblirsch[22] provided data on mortality for bed bugs subjected to very slow rates of temperature increase (0.06 °C min^{-1}) more relevant to whole-room heat treatments, and found that longer times were required to kill bed bugs (e.g. 71.5 min at 48 °C for adult bed bugs) than previously reported (e.g. 60 min at 44 °C for adult bed bugs[38]), which they attributed to the effects of gradual warming. However, Kells and Goblirsch[22] also found that 50 °C (after gradual warming) was lethal for any time period (nymphs or adults), which is completely consistent with the data from the present study (50 °C led to 100% mortality for heat durations of 45 s or longer for adult males) (Fig. 1). Other studies have not found any changes in survival of bed bugs at high temperatures (up to 48 °C) after previous exposure to sublethal elevated temperatures, such as 37 °C for 1 h or 30 °C for 2 weeks.[39] It has been shown recently that adult bed bugs exposed to ‘sublethal’ temperatures (35.5–40 °C) over a period of 3–9 days do have lower fecundity, higher mortality and lower egg hatch rates (especially for the longer periods and higher temperatures), leading Rukke *et al.*[40] to suggest that such exposures might help control efforts by reducing, while not eliminating, bed bug populations. However, a goal of 100% mortality is usually considered to be desirable because heat treatments do not provide residual control,[22] and bed bugs or eggs that escape detection or survive the treatment can ‘set the stage for a population rebound’.[6]

3.2 Lethal exposure times for luggage

Bed bugs on the outside of suitcases were usually killed within 3 min of exposure to 75 °C when they were not hidden in crevices, and within 6 min if protected by piping or seams (Fig. 3). Bed bugs were placed on an exposed area on the suitcase immediately before moving the suitcase into the heated chamber, and most of the bed bugs were successful at finding and inserting themselves into such a crevice: 59% (147/250) of the bed bugs were in crevices (one of those had entered a zippered compartment), and 41% (103/250) were exposed on the surface when the luggage was removed from the chamber. The knockdown results suggest that a bed bug will stop moving within ~15 s after its surroundings reach 50 °C, which probably explains why some bed bugs stopped moving and then died before reaching a crevice. Bed bugs were not observed while they were in the heated chamber, and although their location was noted on the luggage at the time it was removed from the chamber, the exact duration of their time exposed or in a crevice is unknown. This may explain why one out of the 21 exposed bed bugs was still alive after 5 min (Fig. 3); that individual may have spent some of the 5 min partially protected in a crevice,

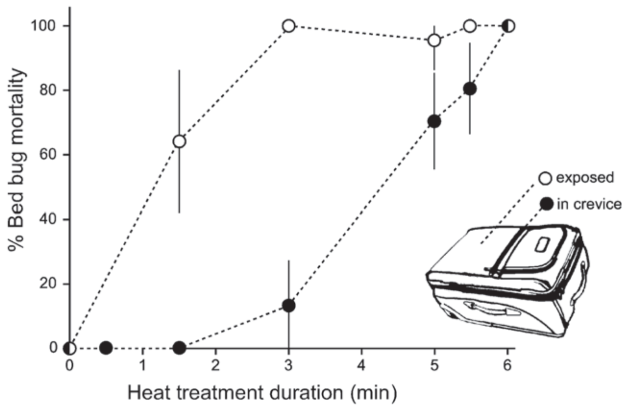

Figure 3. Mortality of adult male bed bugs on luggage placed into a 75 °C chamber as a function of time and location on the surface (exposed versus in a crevice). Each point represents the percentage mortality for a group of at least 18 bed bugs for each time and location combination, for a total of 250 bed bugs (vertical lines indicate 95% confidence limits for proportions).

emerging when the luggage was removed from the heated chamber. None of the 20 control bugs (placed into the same chamber on luggage without heating) died during a 6 min exposure.

The average temperature of the outside of the suitcases measured with a thermal camera was 56 °C within 5 s after being pulled from the chamber (range 46–61 °C, $n = 39$), and so presumably was slightly warmer before the few seconds of cooling. The external temperature was slightly higher after being in the chamber for a longer period of time (Figs 4A and B). Before being placed in the heated chamber, the average temperature of the outside of the luggage was 27 °C (range 24–31 °C, $n = 44$; room temperature range 22–26 °C). There was no significant difference in external temperature between the two different suitcases for any heat treatment duration, and therefore the data for the two suitcases were combined (ANCOVA of external temperature with time duration as a covariate and luggage as a class variable: time duration $F_{1,36} = 40.18$, $P < 0.0001$; luggage $F_{1,36} = 3.04$, $P = 0.09$; homogeneity of slopes test was not significant for the interaction term duration × luggage: $F_{1,35} = 1.14$, $P = 0.29$, and therefore the interaction term was dropped before running the ANCOVA). To put the experimental temperatures in a more familiar context, the temperature of the heated chamber is similar to the maximum temperatures reported for the inside of cars parked in full sun with closed windows in the summer (up to 76 °C,[41] 78 °C[42] or even 89 °C[43]), and therefore the heating of the luggage in the heated chamber would be expected to follow a similar time course that would occur inside a vehicle in such conditions.

Temperature recorders (iButtons) inside the empty luggage recorded an increase in temperature to an average of 40 °C while the luggage was being heated (Figs 4A and 4C) (range of temperature maxima 32–61 °C, $n = 25$; the 61 °C was an outlier, being more than two standard deviations above the mean, and the next highest peak reading was 52 °C). The high level of variability in the internal readings could be partly due to the loose iButtons (Fig. 4C) moving around within the suitcases and ending up in a new position as the suitcases were moved into and out of the chamber. The temperature increase at the center of the luggage would presumably have been smaller had the suitcase been full of items. The internal temperature was slightly higher after being in the chamber for a longer period of time (Fig. 4A). There was no significant difference in internal temperature between the two different suitcases for any heat treatment duration, and therefore the data for the two suitcases were combined (ANCOVA of internal temperature with time duration as a covariate and luggage as a class variable: time duration $F_{1,22} = 5.59$, $P = 0.03$; luggage $F_{1,22} = 1.04$, $P = 0.32$; homogeneity of slopes test was not significant for the interaction term duration × luggage: $F_{1,21} = 0.05$, $P = 0.82$, and therefore the interaction term was dropped before running the ANCOVA). The internal temperature of the luggage immediately prior to being heated again averaged 32 °C (range 22–39 °C, $n = 25$), and therefore had not completely cooled back down to room temperature. In contrast to bed bugs on the outside of luggage, bed bugs inside luggage would pose similar challenges to killing by heat to those reported with bed bugs inside furniture. It would be expected that hours might be required to bring the temperature up to a lethal temperature at all points inside a full suitcase when heated from the outside, similar to what has been found for bed bugs inside furniture.[16,22]

The specific pieces of luggage used in the evaluation were standard popular models of suitcases, covered with fabric that had piping and flaps that covered zippers. No differences were recorded between the two different suitcases (external temperature, internal temperature, movement of bed bugs or mortality of bed bugs), and therefore these values may be typical for this general type of luggage. In order to be on the outside of luggage, bed bugs have to be able to cling to the surface. Bed bugs are able to walk or hang onto fabric or rough surfaces much more readily than hard smooth metal or plastic surfaces.[44,45] Travelers could presumably minimize the possibility of bed bugs clinging to the outside surfaces of their luggage by using hard-sided luggage with few seams or crevices.

3.3 Movement of bed bugs on or into luggage

There is little available information about the ability or tendency of bed bugs to leave or enter luggage, and therefore movement of bed bugs into luggage (across closed zippers) was measured directly. Bed bugs did not move into the luggage (into a zippered compartment) from the outside very often during the heating period. After the luggage had been in the heated chamber (for up to 6 min), only one bed bug out of a total of 250 bed bugs was found inside a zippered compartment, and it was dead. The bed bugs were placed at different starting locations on the fabric on the outside of the luggage; about half of the bed bugs (135/250) were placed within 5 cm of the zipper tabs (there was a small opening between the two tabs even when the zipper was fully closed) before the luggage was placed in the heated chamber. After the heating period, bed bugs were found as far as 20 cm from their initial location, but most (95%) were within 10 cm of their original location.

Similar results were found for luggage at room temperature after leaving the bed bugs for 24 h. Only five bed bugs (out of 170 bed bugs in total) were found inside a zippered compartment at the end of a 24 h period, and this occured during five separate 24 h periods (only one bed bug each time). About a third of the bed bugs (56/170) walked across at least one closed zipper during the 24 h period (inferred from the bed bug starting and final locations) without moving inside the zippered compartment. The light regime did not appear to affect this movement, as three of the five 24 h periods that resulted in a movement into a zippered compartment were in 24 h darkness, and the other two were 16:8 L:D light cycles. An additional five bed bugs (out of 170 bed bugs in total) were never found, either inside or on the outside of the luggage, but there are a large number of hiding places on suitcases (deep seams, wheel wells, etc.). Three of the five missing

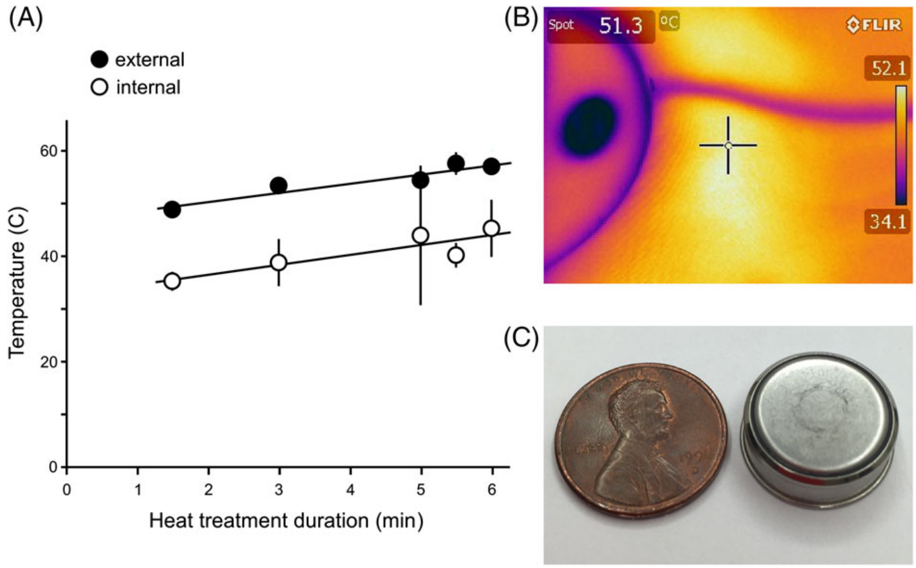

Figure 4. (A) External and internal temperatures of suitcases after different amounts of time in the 75 °C heated chamber (means ± 2SE). The lines show the results from linear regression: external temperature with time, $y = 1.74x + 46.8$, $r^2 = 0.52$, $n = 39$, test for slope of zero $t = 6.3$, $P < 0.0001$; internal temperature with time, $y = 1.89x + 32.7$, $r^2 = 0.24$, $n = 25$, test for slope of zero $t = 2.68$, $P = 0.01$ (y is temperature in °C and x is time in min). (B) Example of thermal image of outside of suitcase after removal from heated chamber. (C) An iButton (with penny on left for scale) was placed inside a suitcase for monitoring internal temperature during heating.

bed bugs went missing during periods of 24 h darkness, and the other two during 16:8 L:D light cycles.

Although the light regime did not affect the movement of the bed bugs on the luggage during the 24 h periods, the age of the bed bugs did. Younger bed bugs were more frequently found inside the luggage than older bed bugs, and were more frequently missing ($P < 0.0001$ for Fisher's exact test, three age groups × three locations, $df = 4$) (Table 1). The missing bed bugs are not driving the observed significant relationship between age and location; either eliminating the missing bed bugs or combining them with the inside category (presumably their most likely location) made no difference in the outcome of the analysis. Although different ages of bed bugs were used in these measurements, all of the bed bugs were adult males. It is unknown how different these results would be for adult females or immature bed bugs, but immature bed bugs are smaller, and therefore might enter a closed zipper more readily.

The conditions (temperature and relative humidity) were very similar inside and outside the luggage during the 24 h periods, and therefore are unlikely to have affected the probability of bugs entering the luggage. The average temperature inside the luggage was 22.6 °C (range of 24 h averages 21.9–23.1 °C, $n = 14$), and the average temperature outside the luggage (and inside the enclosing plastic bags) was 22.7 °C (range of 24 h averages 22–23.1 °C, $n = 10$). The average relative humidity inside the luggage was 48% (range of 24 h averages 31–60% relative humidity, $n = 3$), and the average relative humidity outside the luggage was 54% (range of 24 h averages 46–58% relative humidity, $n = 6$).

Table 1. Number of bed bug adults by age in different locations 24 h after being placed on the outside of luggage at room temperature

	Location after 24 h			
Age (weeks since eclosion to adult)	Outside of luggage	Inside zippered compartment	Missing	Total
1–2	23 (77%)	3 (10%)	4 (13%)	30
4–5	37 (93%)	2 (5%)	1 (3%)	40
>5	100 (100%)	0 (0%)	0 (0%)	100
All ages combined	160 (94%)	5 (3%)	5 (3%)	170

[a] Data were analyzed with Fisher's exact test; there is a significant relationship between age and location ($P < 0.0001$).

4 CONCLUSIONS

Travel and transport of infested items are generally assumed to be responsible for the spread of bed bugs. Bed bugs are reported on airplanes,[46] hotels, trains, boats and cruise ships,[27] travel with us in bags or folds of clothing,[47] are spread in luggage[27,48–51] and are in luggage cargo holds[50] where they could spread on the outside of luggage. Repellents are being considered for use on luggage,[52] and internally heated luggage is now commercially available. It would be useful to have more information on the location of luggage-associated bed bugs: what proportion of the bed bugs are inside versus on the outer surface of the luggage, and the frequency with which bed bugs tend to leave or enter luggage. If bed bugs frequently and readily move in and out of luggage, then their transient location is of less importance, but if their movements between the inside and the outside of luggage are as infrequent as measured for the adult male bed bugs in this study, then their location information becomes more relevant for targeted control efforts. While brief 6 min exposure of luggage to high external temperatures (75 °C) will not kill bed bugs inside the luggage, this kind of heat treatment may be an effective and relatively rapid approach for killing bed bugs located on the outside of luggage. For treatment of large numbers of suitcases that share temporary storage during travel (whether cargo holds or hotels), a long heated chamber with a conveyor belt, with suitcases entering at one end and exiting out the other, is one possible design.

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