Lethal Effects of Heat and Use of Localized Heat Treatment for Control of Bed Bug Infestations

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ABSTRACT   Bed bugs, *Cimex lectularius* L., hide in cracks and crevices in furniture and are difficult to control. The bed bug thermal death kinetics were examined to develop a heat treatment method to eliminate bed bug infestations in room contents. High temperatures caused temporary immobilization (knockdown) of bed bugs even with exposures that did not have lethal effects. Exposure of bed bug adults to 39°C for 240 min caused no mortality; however, as temperatures increased from 41 to 49°C, exposure times that caused 100% mortality decreased. The temperature difference to provide a 10-fold change in the mortality was estimated at 4°C, and the estimated activation energy (E_A) was between 484 and 488.3 kJ/mol. This demonstrates that bed bugs are not more resistant or susceptible to changes in temperature than other tested insects and that the temperatures needed to kill bed bugs are relatively low. In room treatment tests, heat treatment times varied from 2 to 7 h with complete mortality of exposed bed bugs within the treatment envelope created by surrounding the treated furniture with polystyrene sheathing boards. Containment and circulation of heat around the treated material were crucial factors in an efficient heat treatment for bed bug control. The room floor material greatly affected containment of the heat. The tested method for limited heat treatment of furniture and other room contents required equipment costing less than US$400 and provided opportunity for residual pesticide application around the room with minimal disruption in use of treated room.

KEY WORDS   *Cimex lectularius*, heat treatment, control, lethal temperature, bed bug

Bed bugs, *Cimex lectularius* L., have been associated with humans for at least the past 3,500 yr (Panagiotakopulu and Buckland 1999). After a decline in bed bug populations in developed countries after World War II (Kruger 2000, Gangloff-Kaufman and Schultz 2003), a resurgence of these blood-sucking insects have been causing increasing problems (Pinto et al. 2007). Because these insects hide in cracks and crevices (Usinger 1966), detection (Cooper and Harlan 2004), and control can be challenging. This is especially true when bed bug aggregations occur on beds, furniture, and other objects where the application of chemical pesticides may cause problems, both for the pest control industry and for customers not willing to accept close contact with pesticides. A physical control method applied to the room contents would be useful as a tool in the overall management of bed bug infestations as a supplement to crack and crevice insecticidal applications to potential harborages.

Heat treatment of a whole room or building is currently used for control of bed bugs (Kells 2006, Pinto et al. 2007) as well as many other insects especially storage pests (Tang et al. 2007). Temperatures between 44 and 45°C (Doggett et al. 2006) have been cited repeatedly as lethal to bed bugs. However, re-examination of the bed bug thermal death requirements using new methods (Gazit et al. 2004, Johnson et al. 2004) is warranted. Besides the direct effect on the bed bugs, sublethal high temperatures can be detrimental to bed bug symbionts, and consequently prevent bed bug reproduction (Chang 1974).

Heat treatments varying from whole-structure to whole-room to containerized treatments have been used by the pest control industry (Pinto et al. 2007), reportedly with positive results. However, the use of solar radiation as a heat source to kill bed bugs in encased mattresses reportedly did not provide control (Doggett et al. 2006) because the insects were able to move away from high temperature areas. Cost of the necessary equipment, the energy requirements, treatment duration, and other difficulties related to commercially available whole room and building heat treatments have motivated the examination of alternative localized heat treatment for control of bed bugs infestations. The objectives of these studies were to examine the bed bug thermal death kinetics and apply results to the development of heat treatment as a tool to eliminate bed bug infestations in room contents.

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Materials and Methods

Bed Bugs. ICR and Harlan strains of the common bed bug were reared at the University of Florida's Department of Entomology and Nematology (Gainesville, FL). The insects were maintained in 240-ml glass rearing jars (Ball Collection Elite, Jarden Home Brands, Muncie, IN) lined on the bottom with a 90-mm filter paper circle (Whatman no. 1), with harborages made from cardboard (90 by 60 mm) folded in a fan-like manner. To prevent insect escape, nylon mesh with 90-μm opening was placed over the mouth of the rearing jar and secured by a screw-on lid. Bed bugs were maintained at 23–24°C, ≈50% RH, and a photoperiod of 12:12 (L:D) h, and they were fed to engorgement once a week on chicken hosts. Adult bed bugs were used in the experiments because they can be easily manipulated without causing harm to the insects, although eggs may be more resistant (within 1°C) to the effects of high temperatures (Drenski 1928; Mellanby 1935, cited by Johnson 1941). Bed bug colony maintenance is performed under UF/IACUC approval E876.

Lethal Thermal Dose Determination. Polystyrene board was cut to fit inside an analog-controlled temperature water bath (Isotemp model 205, Thermo Fisher Scientific, Waltham, MA). Ten circular holes (≈1.5 cm in diameter) were cut into the polystyrene board to allow Kimax glass test tubes (20 o.d. by 150 mm, Thermo Fisher Scientific) to fit securely, thus allowing about two thirds of each test tube to be immersed in the water. While the water bath was heating, adult bed bugs were separated from the colonies with feather-tipped forceps. Two bed bugs were placed in each 1.5-ml plastic microcentrifuge G-Tubes (Thermo Fisher Scientific). When the water bath reached the desired temperature, bed bugs were quickly transferred from the microcentrifuge G-Tubes to the immersed glass test tubes. The water-bath was heated to 39, 41, 43, 45, 47, and 49°C. Bed bugs were held at each temperature for varying lengths of time between 0.5 and 240 min depending on the temperature, so that a series of four to five treatment lengths causing 0–100% mortality at each temperature could be obtained. Between 114 and 198 insects were tested for each temperature except for 49°C at which only 24 insects were tested at two treatment durations (0.5 and 1 min). Tubes were removed from the water bath at the designated time and bed bugs were transferred to plastic snap-cap vials containing filter paper as harborage. Knockdown was recorded immediately, and final mortality counts were recorded 24 h later. At least 12 insects were used for each temperature/treatment duration combination.

Methods described for determination of thermal death kinetics (Gazit et al. 2004; Johnson et al. 2003, 2004; Wang et al. 2002a,b) were used to determine the effect of different temperatures on bed bugs. Bed bug survival at each test temperature was plotted against exposure times, so that survival curves were obtained. A thermal death curve was obtained by plotting the observed minimum time in minutes [log (time) plotted on y-axis] needed to obtain 100% bed bug mortality at the different exposure temperatures (x-axis). The duration of treatment at different temperatures needed to kill bed bug adults was calculated and used to estimate length of heat treatments to be used in rooms as described below.

Heat Treatment of Rooms: Insects. Bed bugs were separated with feather-tipped forceps into glass vials (15 ml; Thermo Fisher Scientific) also covered with nylon mesh fabric that allowed quick penetration of heated air into vial during heat experiments. Five to 10 live, mixed sex, adult bed bugs were placed in each vial, and three to four vials were placed in different locations among the room furniture to allow estimation of heat penetration and bed bug mortality. Because a successful room treatment would require insect elimination, we did not try to measure differences in bed bug mortality, which would require large numbers of insects. Bed bug mortality was calculated on a per-vial basis; a vial was considered live if at least one bed bug survived, or dead if all insects were killed during treatment. A control vial was maintained in a closet or shelf in the treatment room, away from the heat. Complete survival of bed bugs in control vials was observed in all trials.

Heat Treatment of Rooms: Equipment. Equipment used in the different heat treatment experiments included oil-filled electrical space heaters (model HO-2018, Pelonis Appliances Inc., Grand Prairie, TX; or model EW6507L, DeLonghi, Shelton, CT); box fans (50.8 cm in diameter, Lasko, West Chester, PA); small desktop fans, tape, electrical extension cord, polystyrene sheathing board insulation (122 by 224 by 5 cm, Perma "R", Grenada, MS); 6-mil translucent plastic tarp; and blankets. Temperature monitoring equipment included: outdoor/indoor consumer digital thermometers (Acu-rite, Chaney Instrum. Co., Lake Geneva, WI), temperature recorders (Onset Computer Corporation, Pocasset, MA), and thermocouple probes (model EMTSS-062G-6, Omega Engineering, Stamford, CT) connected to laptop computer running Tracer Daq data acquisition software (Measurement Computing Corp., Norton, MA).

Experimental Rooms. Rooms used in the experiments were all in Gainesville, FL. The room furniture was grouped at the center of the room. Oil-filled heaters were placed on the floor around the furniture and box fans positioned so that air would blow through the radiator of the heaters. Small desk fans were placed on top of the furniture to assist with the air circulation around the treated furniture. A treatment chamber was created around the furniture either with a 6-mil translucent plastic tarp (room D only) or polystyrene sheathing board insulation (all other rooms). Rooms used in the experiments, their contents, and positioning of room contents were as described below.

A bedroom (4.0 by 3.4 m) in an unoccupied one-bedroom apartment in family housing complex at the University of Florida (room D) had a vinyl tile floor over concrete slab, a double bed with bed frame, box spring, mattress, and a headboard, two dressers, a two-seat upholstered sofa and an upholstered chair. A

6-mil plastic tarp was used to create the treatment chamber. For the second trial in this room, four heaters were used. Furniture blankets were use to better contain the heat around the furniture. A bedroom (3.8 by 3.5 m) in an occupied two-bedroom apartment at a commercial rental complex (room M) had a carpeted floor and contained a queen-size box bed with bed frame, box spring, and mattress, a large television and TV stand, a small two-door cabinet, and a floor lamp. Two unoccupied duplex rooms (each $\approx$17.7 m^2) on the second floor in dormitory building at the University of Florida (rooms Ya and Yb) had a vinyl tile floor over concrete and each contained two college-dorm style long twin beds, two desks and chairs, and two dressers. The mattress-supporting frames on the bed were raised to the highest possible position so that dressers, desks and chairs could fit under the beds. A bedroom (4.3 by 3.7 m) in an occupied two-bedroom apartment on the second floor of family housing complex at the University of Florida (room G) had a carpet floor and contained a queen-size box spring and mattress which were raised from the floor with two red clay cored bricks placed at each box spring corner, a large television with cable box and DVD player, two night tables, desktop computer, several plastic bags with clothing, and other belongings.

Except for the bedroom in a two-bedroom apartment in University of Florida family housing complex, which had a minimal bedbug infestation despite previous application of residual pesticides to the perimeter wall/floor junction area, and the mattress and box spring used in the college dorm rooms, all other rooms were not infested with bed bugs.

Data Collection. Temperatures were monitored at various locations within the treated furniture, and at locations where vials with live bedbugs were placed. Temperature probes and recorders were wrapped in blankets and placed inside dresser and desk drawers and other locations to simulate conditions expected to have the slowest temperature increase. Treatments were terminated when the monitoring temperature probes from all locations were above the expected lethal temperature for bed bugs (45°C). At that time, the insulating box or cover was removed, and all temperature probes and recorders were recovered along with any bed bug vials. Vials were labeled appropriately and mortality of bedbugs was evaluated immediately and confirmed in close observation in the laboratory. After the end of trial two in the dorm rooms, the bed bug-infested mattress and box spring were inspected for the presence of live and dead insects. The mattress and the box spring were inspected on external surfaces and the box spring's bottom cover was removed and the underside of the box spring top was also examined thoroughly.

Results and Discussion

Lethal Thermal Dose. Bed bugs exposed to heat suffered high levels of knockdown even at temperatures that ultimately produced low mortality (Table 1). For temperatures between 43 and 49°C, 100% initial knockdown corresponded to 0–100% final mortality, depending on the exposure time. These results demonstrate that short exposures to temperatures above 41°C will cause temporary immobilization of bed bugs, even when lethal levels were not reached. Once the bed bugs' exposures to high temperatures were interrupted, some insects were able to survive. We did not test long-term survival of the heat-exposed bed bugs and did not determine whether survivors' fitness was compromised. Bed bugs that survived exposures to nonlethal temperatures have been shown to have reduced fitness (Janisch 1933, 1935, cited by Johnson 1941). Sublethal effects of high temperatures are documented for several insects (Neven 2000, Mahroof et al. 2005). However, thermal wounding by sublethal temperatures may be as deadly, but without obvious effects that lethal temperatures cause (Denlinger and Yocum 1998).

Coefficients for the $n = 0$ kinetic order model for the thermal mortality of bed bug adults were higher than those for any other kinetic order (Table 2; Fig. 1); therefore, parameters for zero-order equations were used for determination of lethal thermal doses. Exposure of bed bug adults to 39°C for a maximum of 240 min caused no mortality in the exposed insects. However, bed bug survival decreased with increased length of exposure to temperatures between 41 and 49°C (Fig. 1). At 41°C, exposures of 60 min were necessary to cause 11% mortality in bed bugs, but all bed bugs were killed with 41°C exposures of 100 min. At 43°C, 42% of insects were killed with 10-min exposures, 75% with 20-min exposures, and no bed bugs survived 25-min exposures. With 45°C, 2-min expo-

Table 1. Mean percentage of initial knockdown and mean percentage of 24-h mortality ($\pm$ SE) among bed bug adults exposed to five different temperatures for varying lengths of time

Temp (°C)	Time[a] (min)	% knockdown[b] (mean $\pm$ SEM)	% mortality[c] (mean $\pm$ SEM)
49	0.5	100 $\pm$ 0	75 $\pm$ 11
	1	100 $\pm$ 0	100 $\pm$ 0
47	0.5	100 $\pm$ 0	0 $\pm$ 0
	1	100 $\pm$ 0	50 $\pm$ 18
	1.5	100 $\pm$ 0	75 $\pm$ 11
	2	100 $\pm$ 0	92 $\pm$ 8
	2.5	100 $\pm$ 0	100 $\pm$ 0
45	2	100 $\pm$ 0	0 $\pm$ 0
	2.5	100 $\pm$ 0	8 $\pm$ 8
	3	100 $\pm$ 0	50 $\pm$ 13
	3.5	100 $\pm$ 0	83 $\pm$ 11
43	7.5	100 $\pm$ 0	0 $\pm$ 0
	10	92 $\pm$ 8	42 $\pm$ 20
	15	100 $\pm$ 0	42 $\pm$ 8
	20	100 $\pm$ 0	75 $\pm$ 11
	25	100 $\pm$ 0	100 $\pm$ 0
41	60	6 $\pm$ 6	11 $\pm$ 7
	70	50 $\pm$ 18	33 $\pm$ 11
	80	92 $\pm$ 8	67 $\pm$ 11
	90	92 $\pm$ 8	75 $\pm$ 11
	100	100 $\pm$ 0	100 $\pm$ 0

[a] Only time/temperature combinations used in generating mortality curves in Fig. 1 are presented. $n = 12$ for all temperature/time combinations, except $n = 18$ for 41°/60 min.

[b] Knockdown evaluated immediately after exposure to heat.

[c] Final mortality evaluated 24 h after removal from heat.

Table 2. Coefficients of determination (r^2) for kinetic order ($n = 0, 0.5, 1, 1.5,$ and 2) models for thermal mortality of bed bug adults at four temperatures

Temp (°C)	0[a]	0.5	1	1.5	2
41	0.976	0.975	0.935	0.911	0.908
43	0.913	0.861	0.758	0.705	0.697
45	0.946	0.914	0.841	0.794	0.787
47	0.902	0.952	0.981	0.986	0.987
Mean	0.934	0.925	0.879	0.849	0.845

Models used were $\ln(\text{survival}) = -kt + c$, for kinetic order (n) = 1; $(\text{survival})^{1-n} = -kt + c$, for n ≠ 1. Bed bug survival was regressed against exposure time (minutes) at each temperature (Celsius), and values for k and c were obtained from regression curves (Gazit et al. 2004).

[a] The zero-order model was selected for estimation of mortality curves of bed bug adults exposed to different temperatures due to greater and more consistent coefficients of determination (r^2) values.

sures killed only 8% of the insects, but 3.5 min killed 83% of the insects, whereas only 1-min exposure to 47°C killed 50% of the adult bed bugs and all insects were killed with exposure to 47°C for just 2.5 min. All insects exposed to 49°C for just 1 min were killed, and 75% were killed with just 0.5-min exposure.

Although our results confirm that 45°C can kill bed bug adults (several authors cited by Johnson 1941), it is clear that exposures to these temperatures should exceed 10 min to guarantee total mortality (Table 1; Fig. 2). Temperatures at or above 49°C should cause bed bug death in <1 min. Lower temperatures, between 40 and 45°C, also can be used to kill bed bugs, but necessary exposure times increase significantly. However, because temperatures above 39°C are lethal to bed bug adults, as the temperature increases above this potential threshold level, the accumulated heat stress on the insects increases continuously. Thus, mortality of bed bugs may occur with considerably shorter exposures to >41°C temperatures than those estimated from our experiments.

The thermal death curve (Fig. 2) was similar to curve obtained using predicted times for 99.997% mortality generated by the zero-order model (graph not
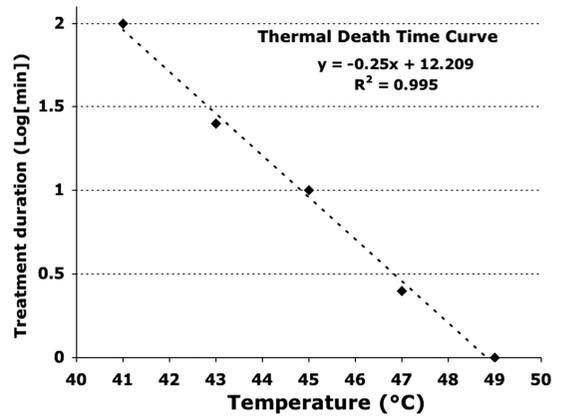
Fig. 2. Thermal death time curve obtained by plotting the observed minimum time in minutes [log(time) plotted on y-axis] needed to obtain 100% bed bug mortality at the different exposure temperatures (x-axis). This curve defines the boundary of time–temperature combinations that can be expected to cause 100% mortality of bed bug adults.

shown). The values for z (the temperature difference to provide a 10-fold change in the mortality) were estimated at 4°C from the thermal death curve, and at 3.97 from the zero-order model. The estimated activation energy (E_A) was 484.4 and 488.3 kJ/mol from the thermal death curve and the zero-order model, respectively. Both z and E_A are similar to those obtained for Mediterranean fruit fly larvae and eggs (Gazit et al. 2004) and codling moth (Wang et al. 2002a). The activation energy for bed bugs falls near the middle of the range for E_A for other insects (Jang 1986; Wang et al. 2002b; Johnson et al. 2003, 2004; Tang et al. 2007), suggesting that bed bugs are not more resistant to changes in temperature than other insects.

Temperature increases needed to kill all bed bug stages are probably below those that would cause damage to furniture and other materials that may be infested with these insects. Previous research (several authors cited by Johnson 1941) demonstrated only a
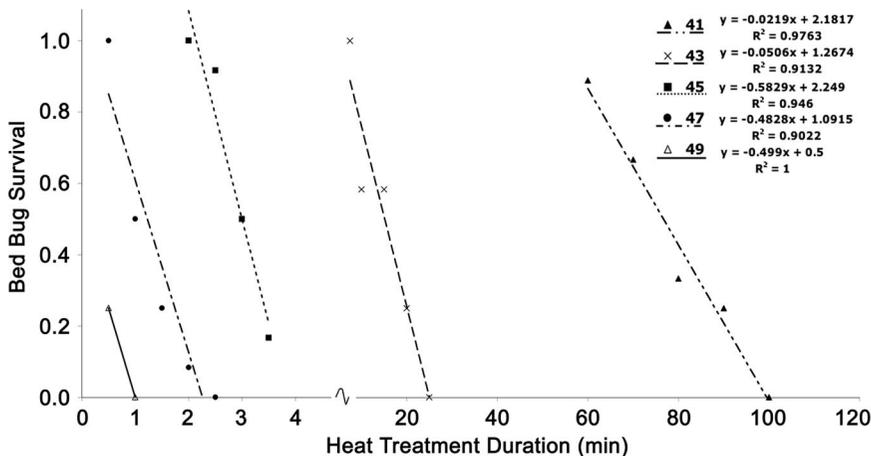
Fig. 1. Mortality curves for bed bug adults exposed to high temperatures (41–49°C) in the laboratory.

Table 3. Heat treatment parameters and observed temperatures (mean and range) in rooms with tile or carpet floor during localized heat treatment of room contents

	Floor type	
	Tile	Carpet
Trials	8	3
Heaters	2 or 4[a]	2
Insulation	Various[b]	Styrofoam boards
Treatment duration (h)	5.7 (4.9–7.3)	2.6 (2.4–3.1)
Room temp. start (°C)[c]	24.0 (21.0–26.6)	25.6 (22.9–25.6)
Room temp. max. (°C)	28.3 (27.7–29.4)	29.2 (26.7–31.7[c])
Lowest max. temp. (°C)[d]	44.7 (37.9–51.8)	46.8 (44.1–51.8)
Highest max. temp. (°C)[d]	58.0 (41.5–67.4)	59.6 (55.4–62.5)
% bed bug mortality[e]	83.4 (0.0[f]–100)	100 (100–100)

[a] Four heaters used in second trial only.

[b] Initial trial, plastic tarp; second trial, tarp + blankets; other trials, Styrofoam boards.

[c] Room temperature at start of treatment and max during treatment. Room temp not recorded for every trial.

[d] Lowest or highest maximal temperature among the all location within the treatment envelope for each trial.

[e] Calculated on a per-vial basis (three bed bug vials per trial each with five or 10 adult bed bugs) so that each vial was considered live if at least one bed bug survived, or dead if all insects were killed during treatment.

[f] Some dead bed bugs in vial placed between mattress and box spring where highest max was recorded.

minimal difference in lethal temperatures between bed bug eggs, nymphs, and adults. To simulate the reported higher lethal temperatures required to exterminate eggs (Mellanby 1935 cited by Johnson 1941), we calculated a 1°C shift to the right in the thermal death curve (Fig. 2). For a fixed exposure temperature, the exposure required for total mortality of heat-resistant life stages increased by ≈80% for each 1°C increase in lethal temperature. Although doubling the exposure time represents significant increase at low temperatures, for temperatures ≥45°C, for which the exposures times are very short (<10 min), these increases are insignificant for practical applications.

Heat Treatment of Rooms. Among all heat-treatment trials, those in rooms with carpeted floors produced lethal temperatures for the bed bugs in the shortest times (2.4–3.1 h), compared with treatment times between 4.9 and 7.3 h for rooms with tile floors (Table 3). Temperatures at different locations within the treatment envelope (Fig. 3) varied depending on the position of the heaters and fans, amount of furniture and other materials within the envelope being heat-treated, and level of insulation between the temperature monitor and the heated air inside the treatment envelope. The difference between the lowest and the highest maximum temperatures measured in each room trial varied from >4 to <23°C. Highest temperatures were always measured at unprotected locations exposed to the heated air in the treatment envelope. Lowest temperatures were observed when temperature monitors were placed inside drawers or other insulated locations, especially when wrapped in blankets that added insulation from the high air temperature in the treatment envelope.

The heating rates for different locations followed trends that also depended on the degree of insulation between the heaters and heated air in the treatment envelope and the temperature-monitoring device. Temperatures at exposed locations tended to increase rapidly in the initial phase of the heat treatment but tended to stabilize toward the final phase of the treatment. In contrast, temperatures in locations insulated from the heated air rose very slowly in the beginning of the treatment, but then reached a higher rate of increase toward the end of the heat treatment. Temperatures at these insulated locations continued to rise even after the heaters had been disconnected, especially when the treatment envelope was maintained intact.

Despite generating temperatures well above the lethal levels for bed bugs (41–49°C) within the treatment envelope, the heat treatment did not elevate the
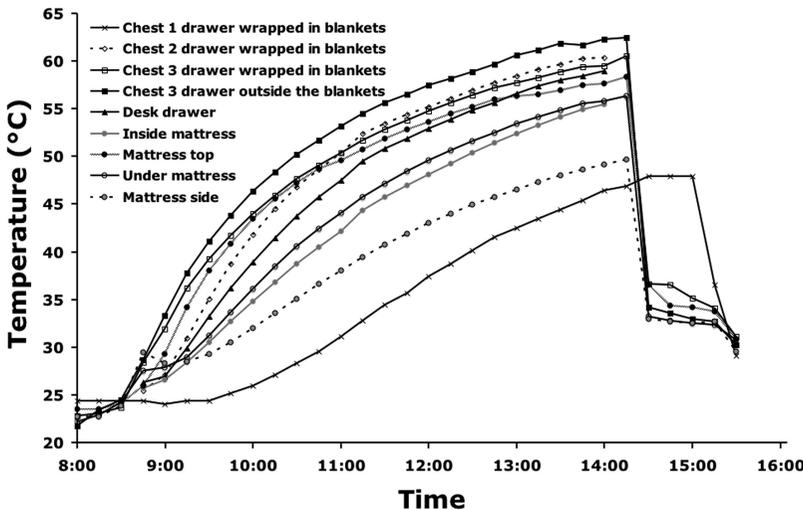
Fig. 3. Temperature at different locations within the heat-treated furniture during trial 2 in room Yb.

room temperature to temperatures $>32°C$ (Table 3). Maintaining the room temperature at comfortable levels for human activity is very important because the heat treatment is intended to supplement a residual pesticide applied to the baseboard and other potential resting areas for bed bugs. Such treatment could be applied while the room furniture is exposed to heat treatment.

The bed bugs placed in different locations during the treatments had 100% mortality for all but two trials: the first trial (room D) and the initial trial in room Yb, both in rooms with tile floors. The initial treatment trial never produced lethal temperatures for the bed bugs in any location where temperature was measured. The maximum temperature reached was $41.5°C$ after 6.3 h, and the total treatment period was 7.3 h, indicating that the temperature in the treatment envelope did not rise during the last hour. This stabilization of the temperature was due to the excessive heat loss through the plastic tarp and the tile floor. Once polystyrene sheathing boards were used as the insulation around the treated furniture, the heat loss was significantly reduced and temperatures continued to increase throughout the treatments.

The only other instance of bed bugs not dying during treatments (one vial in trial one in room Yb) occurred when bed bug vials were wrapped in blankets and placed inside a chest drawer far from the heaters. Because temperature at this location was not monitored from outside of the treatment envelope, heat application was interrupted, based on temperatures at other monitored locations where they reached the desirable level. However this occurred before the temperature of the bed bug vial could reach lethal levels. Discrepancies between the temperatures at monitored locations and this one were because of greater insulation provided by blanket wrapping and poor distribution of temperatures in the treatment envelope. This occurred because both heaters were placed on one side of the insulating box and heated air was not distributed uniformly. These results demonstrate the need to place heaters at opposite corners of the treatment envelope and place fans so that circulation of heated air is maximized.

Our results from heat treatments in rooms also shed some light on results of solar-heat treatment of encased mattresses (Doggett et al. 2006). The effects of both heat loss to the ground or faulty insulation of treatment envelope, as well as the demonstrated need for good circulation of the heated air, were probably factors that caused the ensolarization of the encased mattress not to produce uniformly lethal temperatures.

Containment and circulation of heat around the treated material are crucial factors in an efficient heat treatment for bed bug control. The containment provided by the polystyrene sheathing board insulation was sufficient to prevent excessive heat loss from the treatment envelope and prevent excessive heating of the surrounding room. Because the heat was contained and circulated efficiently, the air space around the treated furniture increased rapidly. This rapid increase of temperature from outside would likely cause bed bugs to move further inside the furniture, moving away from rising temperatures (Doggett et al. 2006). The rapid increase of temperature is also likely to quickly immobilize the bed bugs as observed during our laboratory studies. Also, rapid increase in temperature allows treatment to be completed in a short time, well below the 16 h required for whole-room treatments (Getty et al. 2008). Long treatment times cause major disruption in use of the treated structure, require greater energy input, and prevent simultaneous application of residual pesticides.

The process described herein provides a method for heat treatment of furniture and other room contents, while providing opportunity for residual pesticide application around the room. Total cost for heaters, fans, insulation, temperature monitoring equipment, and other miscellaneous materials used was approximately US$300. Because of the relatively low temperatures required for bed bug elimination, limited heat treatments of room contents using low cost equipment can be accomplished in <6 h. This would allow use of this method in combination with other bed bug control methods with minimal disruption in use of the treated room.

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