Cold Tolerance of Bed Bugs and Practical Recommendations for Control

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ABSTRACT Bed bugs were exposed to freezing temperatures for various exposure times to determine cold tolerance and mortality estimates for multiple life stages. The mean supercooling point for all bed bug life stages ranged from −21.3°C to −30.3°C, with the egg stage reporting the lowest value. A probit analysis provided a lower lethal temperature (LLT_{99}) of −31.2°C when estimates from all life stages were combined, demonstrating that all stages of bed bugs are not capable of surviving temperatures below body freezing and are therefore freeze intolerant. At conditions above the LLT_{99}, bed bug mortality depended on temperature and exposure time at temperatures above LLT_{99}. Based on our model estimates, survival was estimated for temperatures above −12°C even after 1 wk of continuous exposure. However, exposure to temperatures below −13°C will result in 100% mortality in d to ensure mortality of all life stages. Unfortunately, sublethal exposure to lower temperatures did not prevent subsequent feeding behavior in surviving stages. Practical recommendations for management of potentially infested items are discussed.

KEY WORDS freezing, bed bug, supercooling point, cold tolerance, nonchemical control

Bed bugs (*Cimex lectularius* L.) are a significant pest of humans and domestic animals. Changes in traditional pest management practices, insecticide resistance, increased international travel, and lack of public awareness may have attributed to the recent global resurgence of bed bugs (Pinto et al. 2007, Potter et al. 2010). Although bed bugs are not recognized as a disease vector, continuous human exposure may result in depression, anxiety, lack of sleep, and increased sensitivity to their bites (Goddard and deShazo 2009, Reinhardt et al. 2009). Immediately after feeding, bed bugs seek harborage areas within furniture, personal belongings, or other areas near host resting sites to digest the bloodmeal (Usinger 1966). Thus, relocation of used furniture and other personal belongings facilitates the spread of bed bugs throughout society.

Bed bugs cause a substantial economic impact on the affected persons, the lodging industry, property owners, and social and emergency services because of the high costs associated with effective control measures (Miller 2007). Currently, infestations are controlled by conducting frequent inspections either visually, with the aid of monitoring devices or detection by trained canines; laundering; applying steam; heat treatments; and repeated applications of insecticides in areas where bed bugs harbor (Potter et al. 2011). Recent reports demonstrating resistance to pyrethroid insecticides (Moore and Miller 2006, Romero et al. 2007, Steelman et al. 2008) and restrictions on indoor insecticide applications highlight the importance of an effective integrated pest management (IPM) program. An IPM program for bed bugs should include both chemical and nonchemical means for control.

Extreme temperatures have been used to control insect pests in structures and stored products for decades (Fields 1992, Denlinger and Lee 1998). Heat treatments in particular have been used to control bed bug infestations with reported success (Usinger 1966, Pereira et al. 2009, Kells and Goblirsch 2011). Structural heat treatments require special equipment and trained personnel to ensure thorough and safe applications of lethal temperatures (White 2010). Heating equipment used to control bed bugs can be expensive and in some cases, high temperature treatments may degrade or damage equipment or affect protective ratings, such as personal protective equipment used by firefighters, which should not be subjected to temperatures >40°C during cleaning steps (National Fire Protection Association [NFPA] 2008). Unfortunately, persons most at risk of encountering bed bugs include multifamily housing residents, health care professionals, social workers, emergency management services, and so on (Potter 2012). Thus, alternative control options are needed for professionals and the general public involved with control and prevention of bed bugs.

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Exposing bed bug-infested clothing or other small items to freezing temperatures may be a viable control option for people at risk of bed bug infestations. However, information pertaining to the cold tolerance of bed bugs is limited. Benoit et al. (2009) reported 100% mortality when female bed bugs were exposed directly to −16°C for 1 h. Naylor and Boase (2010) recommended exposure to −17°C for 2 h to control both adults and egg stages. Furthermore, contrasting reports recommend exposing bed bug-infested items to temperatures below freezing for at least 2 wk to ensure mortality of all life stages embedded within semiinsulated materials (Potter et al. 2007). More accurate recommendations for freeze treatments would benefit professionals and the general public.

For practical applications, determination of freeze tolerance parameters for all life stages is critical. Two common cold-hardiness strategies used by insects for surviving subzero temperatures include freeze intolerance and freeze tolerance (Salt 1961, Baust and Rojas 1985, Zachariassen 1985, Bale et al. 1989). Freeze-intolerant species generally attempt to protect themselves from freeze injury by lowering the freezing point of body fluids (i.e., supercooling; Bale et al. 1989). Freeze-tolerant species permit freezing of tissues, but prevent injury through a variety of mechanisms (Teets and Denlinger 2013). Evaluating the supercooling point (SCP), and lower lethal temperature (LLT), provides information on freeze tolerance of the target organism (Denlinger and Lee 1998). The SCP measures the temperature at which body tissues freeze, while the LLT evaluates animal survival at short-term exposures to a given temperature. In addition to providing information on freeze tolerance, the SCP and LLT provide initial parameters to further explore exposure time as it relates to lethal temperatures.

For this study, the SCP and LLT were evaluated for all life stages of bed bugs, as well as their potential to feed postexposure to sublethal temperatures. In addition, the relationship between temperature, time, and mortality was explored using a response surface experiment and a prediction model was developed for practical control recommendations. Finally, our model was compared with actual mortality data collected from bed bugs exposed to a variety of general house-hold freezers. These findings provide clear recommendations for the appropriate combinations of temperature and time required to freeze items potentially infested by bed bugs.

Materials and Methods

Insects. Bed bugs were obtained from stock cultures of the ECL-05 *Cimex lectularius* L. field strain (Olson et al. 2009) unless noted otherwise. Colonies were maintained under standard conditions of 25°C and a photoperiod of 14:10 (L:D) h in 16 oz glass jars with folded pieces of filter paper (Fisher 9.0 cm) for harborage and egg deposition. Jars were covered with fabric (Precision Woven Nylon Mesh 193 × 193, McMaster Carr, Chicago, IL) with a pore size of 0.08 μm for ventilation and containment. Colonies were fed weekly using a method similar to Montes et al. (2002) with soon-to-expire stocks of human blood (1 U of Type A red blood cells and 1 U of plasma reconstituted to 600 ml of whole blood) obtained from the American Red Cross (St. Paul, MN). For the experiments listed below, "fed" bed bugs had received a bloodmeal to repletion immediately before exposure and "unfed" bed bugs were starved at least 7–14 d before exposure.

Experiment I: SCP. The objective of this experiment was to determine how SCP varies by fed status and life stage. Surface-contact thermometry was used to establish SCPs following methods of Carrillo et al. (2004). Both fed and unfed adult, fifth and fourth instars ($n = 24$) were individually attached to 24 gauge copper-constantan thermocouples coated with a thin layer of high-vacuum grease (Dow Corning, Dow Corning Corporation, Midland, MI). Because of their reduced mass, third, second, first instars and eggs were attached to a 40 gauge copper-constantan thermocouple. Bed bugs were suspended on the thermocouples inside the center of polystyrene cubes (19 by 19 by 19 cm) with internal temperatures at 0°C and cooled at a rate of −1.0°C/min by placing the cube into a −80°C freezer (Revco model ULT790–5-A31 Kendro Laboratory Products, Asheville, NC). Bed bug temperatures were recorded at a frequency of 9.1 Hz with a multichannel data logger (Personal Daq/56 data acquisition system; Iotech, Inc., Cleveland, OH). To measure SCP, temperatures were recorded to determine the lowest point before the exotherm, which is a slight rise in temperature indicating release from latent heat of fusion, on an otherwise decreasing trajectory (Lee et al. 1992). After the release of heat, the temperature recorded by the thermocouple continues to drop until the thermocouple reaches the lowest temperature in the freezer.

Eggs were tested at two ages, with "young eggs" deposited by females within 48 h before exposure and "old eggs" deposited 3–6 d earlier. Eggs were left attached to filter paper to reduce risk of chorion damage. For accuracy purposes, individual bed bugs and eggs were grouped by Stage and Fed status, but the group order was randomized to avoid frequent thermocouple changes, mixing of thermocouples connected to the data logger, and repeated recalibration of the thermocouple sensors. The SCP results were tested for outliers via the maximum normed residual test for outliers (Snedecor and Cochran 1989). Analysis of variance (ANOVA; Proc GLM) was used to detect differences for the main effects of Stage and Fed Status and their interaction. Mean SCPs were compared by LSMEANS using Tukey–Kramer adjustment (SAS Institute 2009) and reported as means and 95% CIs.

Experiment II: LLTs. This experiment measured mortality across all stages with short-term exposures to LLTs to determine freeze tolerance of bed bugs when compared with SCP values. Individuals of all stages, including both fed and unfed bed bugs, young and old eggs, resulted in a total of 4,320 bed bugs used ($n = 16$ bug stages, 10 bugs per stage, 9 temperatures, and 3

replicates). To evaluate natural mortality, bed bug cohorts were randomly assigned to 24°C during assignment of cold exposure treatments; estimating natural mortality consisted of 320 bugs (16 stages, 10 bugs per stage, and 2 replicates). Bed bugs were transferred into 5-ml plastic vials with a plastic screw-top cap and with an 8-mm hole drilled into it. Filter paper was fit under the cap to prevent escapes and allow for ventilation. Eggs were set up in a similar manner and collected as previously described. Temperatures within the vials were recorded with a 24 gauge copper-constantan thermocouple (Eaton and Kells 2011). For each exposure, vials containing specific Stages and Fed Status were randomly assigned to temperatures: 0, −5, −10, −15, −20, −25, −30, −35, and −40°C. Similar vials containing bugs for controls were randomly assigned throughout the trials and maintained at 24°C. Vials were placed in the center of polystyrene cubes (0.35^3 m^3) with a starting temperature of 0°C and were cooled at a rate of 0.3–0.4°C/min. Vials were removed when the target temperature was achieved and immediately placed into slowly elevating temperatures (i.e., −25°C, −15°C, 0°C, and 5°C, 10 min each) to acclimate the bed bugs back to room temperature (24°C). Bed bugs were relocated to plastic Petri dishes (50 by 9 mm, Falcon 35 1006, Becton Dickinson Labware, Franklin Lakes, NJ), with filter paper (Fischer Scientific, P5) and fabric mesh for ventilation. Adult and nymph mortality was assessed 24 h postexposure by gently agitating the bug with a forceps to observe movement. Unassisted movement of any appendage during the assessment period was reported as survival. Egg mortality was observed every 24 h for a minimum of 10 d. Remaining nonhatched eggs were considered dead 10 d after the last egg in that Petri dish had hatched. Analysis of the LLT was conducted using a Proc Probit with a logistic distribution option (SAS Institute 2009). This method enabled us to assess proportion mortality against temperature and calculate $LLT_{50,99}$ estimates by stage and their associated 95% CIs.

Seven days postexposure, surviving stages were combined by temperature treatment and offered a warmed blood diet using a modified artificial feeding system to evaluate sublethal effects on feeding behavior. The modified system consisted of a 16-ml plastic test tube that contained 1–2 ml of blood warmed in a warm-water bath then covered with stretched Parafilm (Alcan Packaging, Peachtree City, GA). The test tube was inverted over the screened dish containing the bed bugs on filter paper. Bed bugs were allowed 15 min to climb the filter paper harborage to reach the bloodmeal and feed. The data were analyzed by ANOVA (Minitab 2010) and reported as mean percent fed.

Experiment III: Estimated Mortality Based on Temperature and Exposure Time. The relationship between temperature and time was expected to be nonlinear based on the previous results from Experiment II. Therefore, a Central Composite Design (CCD) with 21 combinations of temperature and time was generated by Design Expert version 8.0.1 to explore the relationship between temperature and exposure time on bed bug mortality. The temperature inputs were bound by −30°C as the lower estimate and 0°C as the upper estimate based on the LLT data. Exposure times were bound for practical reasons by 2 and 168 h. The CCD design used the axial inputs (−30°C, 0°C; 2 h, 168 h), identified two additional factors (−25°C, −5°C; 26 h, 144 h) and a center data point (−15°C; 85 h) to produce 21 total combinations of temperatures and times necessary to create the model. Specified temperatures (within 2–4°C) were achieved using temperature control cabinets (Percival Scientific, model: 130BLL, Perry, IA) or Styrofoam boxes (25 cm^3, 3 cm thick) placed in a Gibson (model: FV21M2WKFA; Appliance Corporation, Greenville, MI) freezer. Temperatures were monitored using calibrated HOBO data loggers model #U23-002 (Onset Computer Corporation, Bourne, MA).

Groups of 20 eggs (0–7 d old), first instars, fifth instars, males, and females (both fed and unfed stages) were exposed to each predetermined combination of temperature and exposure time. Adults, nymphs, and eggs were placed separately into modified Petri dishes as previously described and cooled (5°C, then 0°C; 10 min each) before placement in each freezer and slowly warmed (0°C, then 5°C; 10 min each) before removal. Eggs remained attached to the original filter paper during both the exposure and the recovery periods.

A second CCD and set of mortality data were generated to confirm the initial model estimates. The second CCD design included axial inputs (−12°C, −20°C; 24 h, 96 h based on the previous CCD results), identified two additional inputs (−13°C, −19°C; 34.5 h, 85.5 h), and a center data point (−16°C; 60 h) to produce another 21 combinations of temperature and time treatments. The previous CCD analysis showed differences in mortality estimates between eggs and other life stages, but not fed status. Therefore, only eggs and unfed first instars were used in the second CCD to limit the number of treatments. For the second CCD data set, unfed first instars and eggs (0–7 d old) were housed in 1.5-ml centrifuge tubes (Fisher, Pittsburg, PA) and placed within an aluminum heating block (described in more detail by Eaton and Kells 2011) to maintain precise temperature settings (within 1–2°C) throughout the duration of the experiment.

Mortality for adult and nymph stages was assessed 24 h postremoval from the freezer. Egg mortality was recorded as the percent of un-hatched eggs 1 wk postexposure. The data were analyzed by ANOVA and a Tukey multiple comparison test was performed to determine significant differences between life stages and fed status using a general linear model in Minitab 16 (Minitab 2010) on arcsine–square-root transformed mortality data. Estimated mortality was plotted as a function of temperature and exposure time.

Experiment IV: Actual Mortality Using Standard Household Freezers. The objective of this experiment was to compare our model estimates to actual bed bug mortality using general household freezers. Three

standard household freezers were used in our analysis including a Whirlpool (model: ET18PKXGW01; Whirlpool USA, Benton harbor, MI), a GE (model: GTS18TBSAWW; General Electric Company, Louisville, KY), and a Kenmore (model:253.21111103; Sears, Roebuck and Co., Hoffman Estates, IL). Each freezer was maintained under normal use conditions (with at least one-half of the space filled and the freezers were opened several times each day) during the experimental period. Freezer temperatures were recorded using HOBO data logger models #UA-002-08 or #U23-002 (Onset Computer Corporation, Bourne, MA) every 10 min for the duration of the study.

A susceptible laboratory strain (Fort Dix) from Rutgers University was used for this experiment. Previous results showed that fed status did not have a significant impact on mortality; thus, bugs were fed 7–21 d before the experiment using an artificial feeding system similar to the one previously described. Groups of five adults and five (third to fifth) instars were placed into each 3.7 cm in diameter and 1.25 cm tall Petri dish. Five replicate dishes were prepared for each exposure period, wrapped together in a thick cotton sock and placed within a 1-gallon plastic bag. Five sets of dishes were placed in each freezer, and one dish was taken out of each bag 1, 2, 3, and 5 d postexposure. Control dishes were set up the same and exposed to room temperature throughout the duration of the experiment. Immediately postexposure, the cotton sock was removed and all dishes were exposed to room temperature conditions. Mortality for each set of bugs was examined as described above 24 h postremoval from the freezer. Mean mortality and freezer conditions including mean, SE, and range were calculated and analyzed in Minitab 16 (Minitab 2010).

Results

Experiment I: SCP. The main effect of Stage was significant ($F_{8,324} = 44.19$; $P < 0.0001$), but not the main effect of Fed Status ($F_{1,324} = 1.33 \times 10^{-3}$; $P = 0.9709$). The interaction of Stage and Fed Status was significant ($F_{6,324} = 7.30$; $P < 0.0001$). A review of simple effects showed that Fed Status was only significant among first instars; thus, SCP estimates for fed and unfed individuals were combined for the remaining life stages (Fig. 1). The youngest stages provided the lowest SCPs, with no significant differences ($P > 0.4139$) between young eggs at −30.3°C (95% CI = −30.7, −29.8), old eggs at −28.1°C (−28.7, −27.5), and un-fed first instars at −28.4°C (−29.29, −27.51). Interestingly, SCP increased significantly ($P = 0.0002$) postfeeding by first instars to −25.0 (−26.2, −23.7)°C. The SCPs of males (−24.3 [−25.4, −23.2]°C) and females (−22.4 [−23.3, −21.5]°C) were significantly different from each other ($P = 0.0075$), but there were no significant differences in SCP estimates between second through fifth instars and females ($P > 0.1603$). The SCPs for males were not significantly different from first-instar fed nymphs, fourth, and fifth instar nymphs ($P > 0.1526$; Fig. 1).

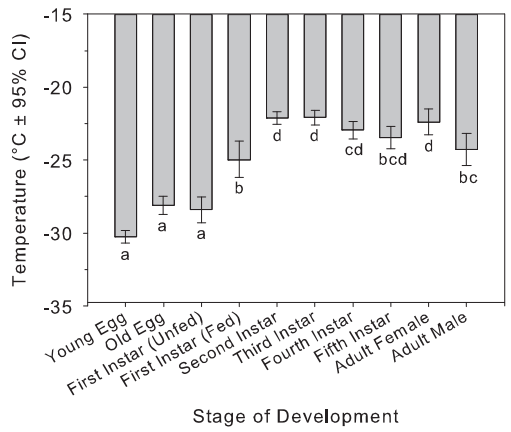

Fig. 1. SCPs for males, females, nymphs, and eggs. For stages other than eggs and first instar nymphs, the SCP values for fed and unfed bed bugs were combined. Mean SCP (± 95% CI) are displayed for each life stage. Different letters denote statistical difference among means ($\alpha = 0.05$).

Experiment II: LLT. The effect of short-term exposure to lower temperatures resulted in two significantly different LLT_{99} estimates for eggs compared with all other bed bug life stages (Fig. 2). Preliminary analysis by stage of slope and intercept estimates and

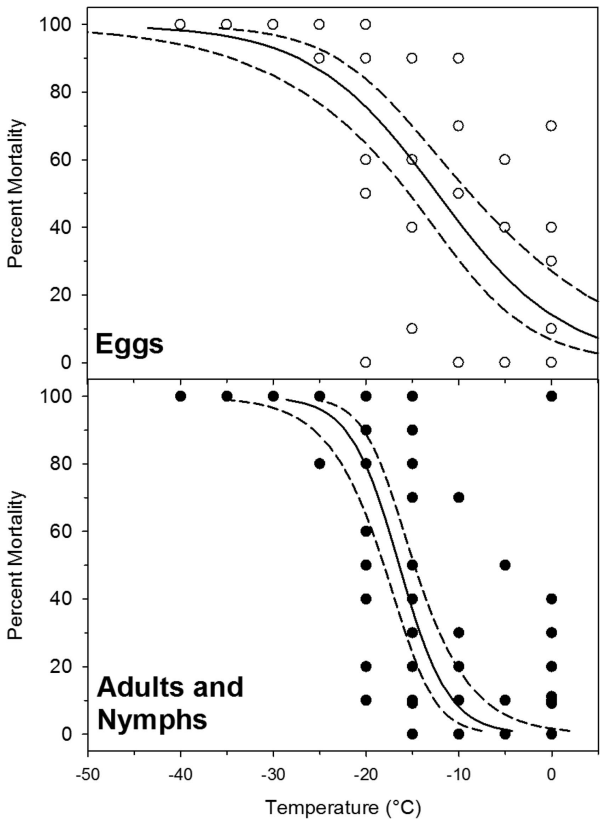

Fig. 2. Mean percent mortality (——) ± 95% CI (— —) of *C. lectularius* for eggs (top) and combined stages of adults and nymphs (bottom) subjected to short-term exposures to LLTs. The Logistic regression characteristics are $\text{Logit}(\hat{p}) = -1.8081 - 0.1473\times$ for eggs and $\text{Logit}(\hat{p}) = -6.2172 - 0.3782\times$ for the adult and nymphal stages.

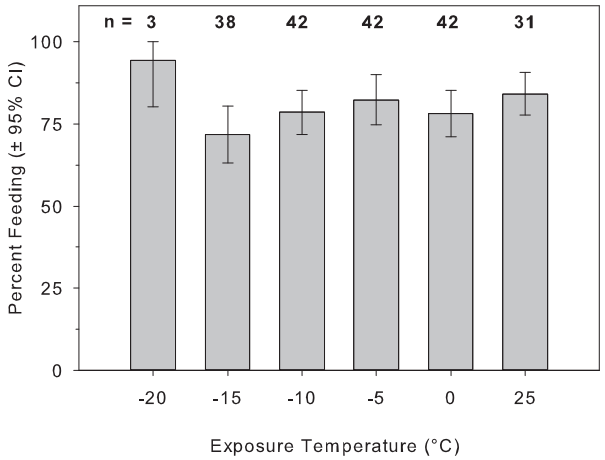

Fig. 3. Mean percent fed (±95% CI) of surviving *C. lectularius* 1 wk postexposure to LLTs. Bolded numbers above each bar indicates the number of individuals surviving the freezing conditions and used in the feeding trial.

their related 95% CIs indicated similarity in mortality curves that enabled formation of these two groups. Data for both young and old eggs were combined and resulted in a slope estimate of −0.1473 (95% CI = −0.1939, −0.1006) and LLT_{99} of −43.5°C (−58.0, −35.9°C). All other life stages combined resulted in an estimate of −0.3782 (−0.2552, −0.5013) and LLT_{99} of −28.6°C (−34.8, −25.3°C). The goodness-of-fit test from the Probit (logistic) analysis was significant ($P < 0.0001$), and variances were multiplied by a heterogeneity factor for both. For eggs, CIs were calculated using a t value of 2.01, and the analysis for other stages incorporated a t value of 1.97 (PROC Probit, SAS Institute 2009). Despite excess variability in mortality at sublethal temperatures, the data did not show a systematic departure from the model estimates. Average natural mortality across all treatments was <3.0%.

Owing to the relatively few survivors at some of the cooler temperatures, life stage, and prior fed status was ignored in the following analysis. The percent of bed bugs that fed postexposure to 0.0, −5.0, −10.0, −15.0, and −20.0°C did not differ significantly from the control group ($F_{5, 192} = 1.54$; $P = 0.18$; Fig. 3). Regardless of exposed temperature, a minimum of 71.77% of the bed bugs fed postexposure to sublethal temperatures. Thus, sublethal exposure to freezing temperatures did not affect the surviving bugs' ability to forage and feed.

Experiment III: Estimated Mortality Based on Temperature and Exposure Time. Excluding eggs, there were no significant differences in mortality estimates between fed and unfed life stages ($P = 0.40$). In addition, there was no significant difference between estimated mortality of adults and nymphs ($P = 0.08$); thus, the data for these life stages were combined (Fig. 4A) into a single surface plot showing estimated bed bug mortality for various combinations of temperature and exposure times. Predicted estimates for egg mortality varied slightly indicating that eggs are more susceptible to colder temperatures compared with other life stages, when time is included as a factor (Fig. 4B). Both models were statistically significant, with adults and nymphs ($P < 0.001$; $R^2 = 0.997$) and eggs ($P < 0.001$; $R^2 = 0.849$). However, the difference between egg mortality estimates and the other life stages may be attributed to natural mortality of the egg stage. On average, 20% of eggs in the control groups did not hatch. Therefore, the arc-sin square root percent mortality model for adults and nymphs provides the best estimate of bed bug mortality for all life stages and is defined as:

$$\begin{aligned} Y\ (\text{arc-sin square root proportion mortality}) = \\ -0.51 + [(4.41 \times 10^{-4}) \times \text{Temp}] + \\ [(2.24 \times 10^{-2}) \times \text{Time}] + [(-1.22 \times 10^{-3}) \times \\ \text{Temp} \times \text{Time}] + [(2.82 \times 10^{-3}) \times \\ \text{Temp}^2] + [(-1.69 \times 10^{-4}) \times \text{Time}^2] + \\ [(-6.85 \times 10^{-5}) \times \text{Temp}^2 \times \text{Time}] + \\ [(-4.65 \times 10^{-6}) \times \text{Temp} \times \text{Time}^2] \end{aligned}$$

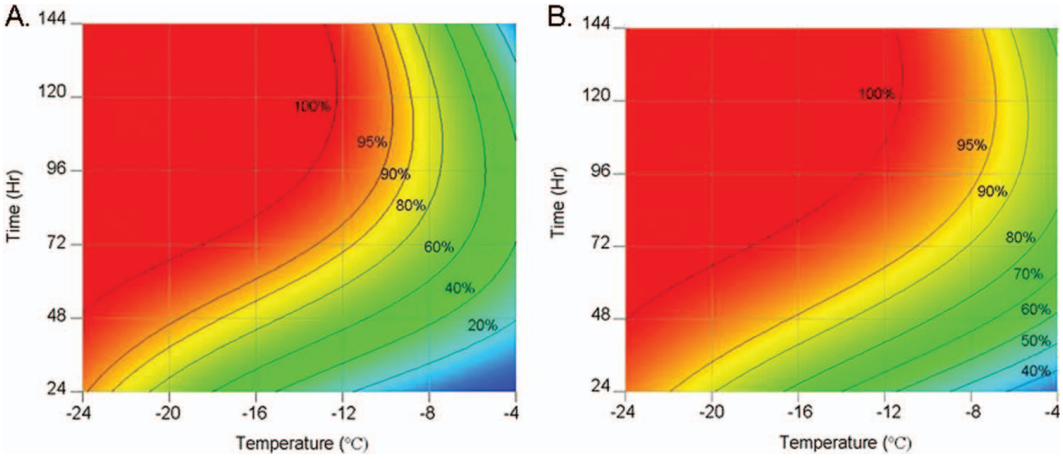

Fig. 4. Response surface plots showing predicted percent mortality relative to temperature (°C) and exposure time (h) for (A) adults and nymphs and (B) eggs of *C. lectularius*.

Table 1. Standard operating conditions and mean mortality of *C. lectularius* after 24-h exposure to three common household freezers

Brand	Operating conditions (°C)				% mortality after 24 h	
	Mean	SE	Range	Defrost cycles/d	Treated ± SE	Control ± SE
Kenmore	−13.7	0.05	(−3.4, −24.1)	14	100.0 ± 0.0	2.0 ± 0.0
Whirlpool	−14.2	0.05	(−7.3, −21.1)	18	100.0 ± 0.0	0.0 ± 0.0
GE	−15.1	0.04	(−10.8, −19.5)	19	100.0 ± 0.0	0.0 ± 0.0

The second CCD experiment performed with first instars and eggs only validated the initial CCD experiment with both models in agreement in terms of the significant factors and their coefficients ($R^2 = 0.741$; $P = 0.001$). Based on the equation above, temperatures higher than −12°C are not practical for effective control measures against bed bugs. A minimum exposure time of 85 h at −15°C is required for 100% mortality of all life stages and exposure time decreases for temperatures below −16°C.

Experiment IV: Actual Mortality Using Standard Household Freezers. The average daily temperature for each freezer was consistent at −13.7°C, but frequent warming, cooling, and defrost cycles resulted in bugs being exposed to a broad range of temperatures (Table 1). Even on the warmest temperature setting, there was a large fluctuation in daily temperatures resulting in a relative SD of 14.5–15.9%. Thus, each group of treated bugs may have been exposed to temperatures as low as −24°C during the first day. There were significant differences ($P < 0.001$) in bed bug mortality between the freezer-treated bugs and the controls after only 24 h exposures (Table 1). Similar mortality rates (100%) were recorded for each freezer at 2, 3, and 5 d (data not shown).

Discussion

This study found that bed bugs were less susceptible to freezing temperatures than previously reported. Benoit et al. (2009) reported −16°C for 1 h; and Naylor and Boase (2010) referenced −17°C for 2 h. In our study, bed bugs survived lower temperatures, with eggs surviving in short-term exposures (LLT) to temperatures as low as −25°C (Fig. 2). When time was considered, 100% mortality of bed bugs occurred after 80 h at −16°C (Fig. 4). Differences in results may be attributed the source of blood used. The two studies referenced above used rabbit or chicken blood, and this study used human blood. Differences in results may also be attributed to the slower rates of cooling and warming that bed bugs were subjected to in this study. While Naylor and Boase (2010) illustrate a cooling rate between −0.5°C/min and −0.13°C/min (approximately), both studies, including Benoit et al. (2009), restored bed bugs to room temperature without intermediate warming steps. In our study, bed bugs were temporarily exposed to intermediate temperatures of 0 and 5°C before freeze treatments and again after treatment before placement into room temperature conditions.

In freezing studies, precooling and postwarming rates can affect survival (Bale 1987, Bale et al. 1989), and brief exposures to chilling or intermediate freezing conditions can elicit a variety of protective responses in insects resulting in rapid cold hardening (Czajka and Lee 1990, Chen and Denlinger 1991). In practical applications, equipment type, insulative properties of items being frozen (Strang 1997), and postfreezing handling procedures can affect the rate of cooling and warming items. Our study evaluated freeze tolerance and mortality using a temperature profile that would likely be encountered during practical application of low temperature treatments. In the future, evaluating different cooling and warming rates may help further refine the models and improve practical recommendations for nonchemical control of insects.

The LLT mortality shows high variability resulting in a correction factor added to the estimate of the 95% CIs. This type of variability has previously occurred in nonchemical control with mold mites (Eaton and Kells 2011) and webbing clothes moths (Brokerhof et al. 1992). This variability may be a factor of target site nonspecificity of cold injury and the diversity of physiological, biochemical, and gross anatomical systems that may be affected by cold temperatures as described in more detail by Teets and Denlinger (2013). Although it is common to graphically present data as a mean and SEM, presenting data from the raw trials in this form illustrates the risks of not properly achieving critical conditions for insect mortality.

Freezing potentially infested items from museum collections or stored food commodities has been a common pest management practice for several decades (Strang 1992). Recommendations for management of museum and stored product pests are vauge, suggesting temperatures treatments below −20°C for several minutes or up to 1 wk (Strang 1997, Fields 1992). According to our results, temperatures below −15°C are sufficient to control all life stages of bed bugs after only 3.5 d; and temperatures below −20°C require ≤48 h. Thus, our results provide more accurate and precise recommendations for nonchemical control of bed bugs using freezing temperatures.

Bed bugs feed every 2.5 d on average, but it may be weeks and even months between available bloodmeals (Reinhardt et al. 2010). Therefore, the amount of blood in the insect gut can vary substantially in field populations. However, fed status had no impact on cold tolerance or mortality estimates of bed bugs. This was surprising because insect diet and gut contents have influenced cold tolerance parameters of several other insect species (Salt 1953, Carrillo and Cannon 2005). It is likely that the bloodmeal did not synergize ice nucleation as temperatures declined and this re-

sulted in no observable differences in SCP, LLT, and mortality estimates based on fed status. For control purposes, it is important to note that fed status of bed bugs does not significantly impact cold tolerance or freezing recommendations.

Some insects have developed a variety of behavioral, physiological, and biochemical adaptations to survive freezing (Storey and Sorey 1996). However, based on the SCP and LLT estimates reported here and by Benoit et al. (2009), bed bugs are not freeze tolerant because LLT mortality data were within the 95% CI for the SCP estimates (Lee et al. 1992). Freeze tolerance would have been suspected if the SCP was substantially higher than the LLT estimates (Bouchard et al. 2006) or if a significant proportion of individuals survived temperatures below the SCP estimate (Eaton and Kells 2011).

If lethal temperatures and exposure times are not maintained during the control period, surviving stages are capable of feeding posttreatment (Fig. 3). Although this study did not evaluate physical damage to the integument or posttreatment effects on reproductive behavior, bed bugs offered a bloodmeal were required to climb a short distance to initiate feeding; thus, host-seeking behavior remained intact. Acquiring a bloodmeal on a regular basis is important for survival, development, and reproduction of bed bugs (Mellanby 1939, Davis 1955, Reinhardt and Siva–Jothy 2007). Thus, failure to maintain lethal combinations of temperature and exposure time during the freezing process may result in continuity of the pest activity and development.

There were significant differences in bed bug mortality recorded from the freezer exposures compared with our model estimates. The exposure time difference between estimated and actual mortality (3.5 vs. 1 d at −14°C) is unlikely attributed to differences between *C. lectularius* strains and more likely caused by large fluctuations in operating temperatures caused by the automatic defrost cycles in the domestic freezers (Table 1). A difference in cold tolerance estimates between strains has been reported in *Calliphora vicina* Robineau–Desvoidy, 1830, a species of blow fly (Hayward and Saunders 1998). However, differences were attributed to geographical origin of each strain. As a structural pest that generally reproduces indoors under consistent environmental conditions, the origin of the strain is unlikely to cause significant differences in mortality estimates.

The extremely low temperatures recorded during the first 24 h in each domestic freezer were likely the cause for different mortality estimates observed between our model and the freezer data collected in Experiment IV. Although our model likely over-estimates the exposure time required for 100% mortality of bed bugs, our model data were collected under controlled conditions with restricted fluctuations in operating temperatures and, therefore, a more reliable source for pest management recommendations. Furthermore, our model applies to situations where automatic defrost cycles may not be installed, such as commercial freezer equipment. Freezers with large fluctuating defrost cycles are generally not recommended for pest control purposes, as temperatures may rise to levels above freezing for the target insect pest (Florian 1990). In addition, homeowners interested in bed bug control methods may not have access to more sophisticated systems and therefore, should follow temperature and exposure time estimates provided by the model.

When alternative control methods are not feasible, bed bug-infested items can be placed in a freezer to destroy all life stages. Items suspected of infestation should be bagged before placement in the freezer to prevent bed bugs from exiting the items and perishing elsewhere inside the freezer. Bagging an item before placing it in a freezer will also protect it against changes in condensation or damage caused by moisture. Infested items should be placed in the freezer at −17.8°C (0°F) for a minimum of 3.5 d, though time may be decreased to 48 h if temperatures average below −20°C. Standard upright household freezers are typically set to −17.8°C (0°F) or lower for proper food storage (U. S. Department of Agriculture–Food Safety and Inspection Service [USDA–FSIS] 2010), though older equipment may not be capable of maintaining this temperature. Insulated items may take 1–3 additional hours to reach lethal temperatures (Carrlee 2003). An indoor and outdoor thermometer should be used to verify operating temperatures before and during the treatment process.

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