Fundamental Research on the Efficacy of Heat on Bed Bugs and Heat Transfer in Mattresses

(PERC docket 12221)

Applying Heat from a Propane-Supplied Heater to Control Bed Bugs In Urban and Commercial Structures

Optimizing Delivery of Heat and Addressing Insect Behavior to Ensure Maximum Efficacy

Final Report
September 2007 – April 2009

Submitted to:

Greg Kerr
Director, Research & Development
Propane Education & Research Council

Submitted by:

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EXECUTIVE SUMMARY

A) PROJECT MANAGEMENT

i) Temp Air, Inc; Burnsville, MN:

Dr. Raj Hulasare, Senior Scientist & Product Manager, Temp Air managed this project as Principal Investigator starting from September 2007 till the completion of the trials in January 2009. The analysis and final report was received in March 2009 for Dr. Stephen Kells, Assistant Professor, Department of Entomology, University of Minnesota, St. Paul, MN.

ii) University of Minnesota (U of M):

Dr. Stephen Kells is the Principal investigator working as Assistant Professor at Department of Entomology, University of Minnesota, St. Paul, MN. Dr. Kells is one of the leading bedbug researchers in the US and often speaks to the public on bed bug issues. He has deep insight into bed bug issues and the problems in controlling the infestation of bed bugs (http://bedbugger.com/2008/04/03/more-bed-bug-research-stephen-kells-at-the-university-of-minnesota/). The link (text partly reproduced as Appendix A) mentions how he ‗dipped bed bug in insecticide and see it live for four days and lay eggs!‘ and also the research funded by PERC. Dr Kells also worked with Dr. Kevin Janni, Department of Biosystems and Agricultural Engineering at the University of Minnesota to investigate engineering aspect of heat transfer through various materials in a space being heat treated (apartment/house).

B) PROJECT TIMELINE

Originally, the research work was planned to start in January 2007 at University of Minnesota. However, number of issues about the contractual details and fund disbursement came up with the University of Minnesota administration and were conveyed to PERC. A final Research agreement from the University of Minnesota was received on September 27, 2007 and the research was initiated in the same month. The last investigative study on re-heating trials in the laboratory was completed in January 2009 and the final report was received in March 2009 to Temp Air (U of M Report: Pages 1-48).

C) THE PROBLEM

Recently, there has been tremendous resurgence of bed bug infestations all over the country. Residences, apartments, low income housing groups, hospitality industry (motels/hotels), property management groups and public spaces like the , hospitals, university dorms, schools have all been battling the bed bug menace. The evolution of resistance to residual insecticides due to repeated usage has contributed significantly to the reemergence of bed bugs as a serious pest and a threat to public health.

Management of bed bug infestations can be an on-going and expensive undertaking using inspections and treatments. Using chemical insecticides has been met with reluctance over safety concerns and risk of resistance development.

As an alternative approach, the use of non-chemical control measures such as those employing high temperatures have demonstrated practical results. Heat treatment of structures offers another relatively safe control mechanism to which bed bugs cannot easily acquire a biochemical resistance.

This project investigated *two major aspects* on efficacy of heat to control bed bugs:

i) *Fundamental research*: temperature versus time mortality investigations of bed bugs to understand their behavior and movement in laboratory
ii) *Applied research*: Applied aspect of heat transfer in structures considering the rate of temperature rise, heat transfer for different materials and its impact on bed bug behavior

D) OBJECTIVES

Considering the basic and applied aspects of applying high temperatures to control bed bugs, *four objectives* were defined for this project (U of M report: Page 5):

1. Determine lethal temperature / time for control of bed bugs.
2. Determine threshold behavior and subsequent movement of bed bugs in response to increasing temperatures delivered through conduction.
3. Determine threshold behavior and subsequent movement of bed bugs in response to increasing temperatures delivered through convection.
4. Determine the rate of penetration of lethal heat through mattresses, upholstered furniture, exterior walls and other structural elements in two habitat types (apartment, house)

E) RESULTS

A comprehensive report submitted by Dr. Stephen Kells, Assistant Professor, Department of Entomology, University of Minnesota, St. Paul, MN is attached (Pages 1 to 48). The report comprehensively discusses the objectives, protocols, results and conclusions. Each objective is expanded in individual sections of methods, results, and conclusions. Recommendations for future work that builds on the findings of present work have also been made separately. Summarized below are the four objectives with brief description of protocol, variables considered and major findings.

1. *Objective 1 - Determine lethal temperature / time for control of bed bugs*:

Set up and variables: The survival and mortality of adults and life stages of bed bugs was investigated in controlled environment chamber with constant rate of temperature increase of 3.6°C/h. The temperature was set at 30, 35, 40, 43, 45, 50, and 55°C with exposure times of 2, 10, 20, 40, 60, 90, and 120 min. Protocol for determining the mortality and emergence of next generation (from eggs if they hatched) is described in the U of M report (Pages 8-9).

Results: Complete mortality (100%) was obtained at temperatures 48, 50, and 55°C for all exposure times except 10-min at 48°C (90%) (U of M report: Page 9). The emergence of nymph, indicative of efficacy of heat to kill was 0 to 10% at temperatures above 48°C for 20 minutes. Even if some nymphs emerged, they did not develop at temperatures higher than 48°C.

Conclusion: It is important to achieve and maintain temperatures of above 48°C for more than 20 min to effectively kill all life stages of bed bugs. In practice, considering the clutter in the treated space and need to penetrate cracks and crevices, the treatment times are much longer ranging from 6 to 8 and hence 48°C would be highly effective.

2. *Objective 2 - Determine threshold behavior and subsequent movement of bed bugs in response to increasing temperatures delivered through conduction*:

Set up and variables: Adult bed bug behavior and movement was observed on a conduction arena comprising of 1 m^2 x 3 mm thick aluminum plate. A ceramic heater placed under the plate heated the plate at the rate of 5.24°C per minute and the average temperature ranged from 27.2 to 42.9°C (U of M report: Tables 2.3, 2.4. Page 28) with a minimum and maximum temperatures of 26.8 and 49.0°C, respectively (U of Report: Tables 2.3. 2.4 under 95% confidence interval).

Results: Initial movement of bed bugs started at average temperature of 27.2°C and feeding behavior was observed at average temperature of 35.2°C. Escape behavior was initiated at average temperature of 41°C. However, post-escape behavior showed bed bugs returning to areas of harborage or areas of lower temperatures to escape heat exhibiting negative taxis (movement from warmer to cooler temperature areas).

Conclusion: Bed bugs tend forage or feed as temperatures rise and the narrow temperature differential between foraging/feeding and escape threshold is indicative that they stay within the arena or are contained. Eventually, with temperatures approaching 48°C and above, they are killed.

3. *Objective 3 – Determine threshold behavior and subsequent movement of bed bugs in response to increasing temperatures delivered through convection*:

Set up and variables: Adult bed bug behavior and movement was observed in conduction arena comprising of a box (1 m x 60 cm x 20 cm high) with aluminum walls, Plexiglas lid and a plywood floor coated with epoxy paint. The arena was divided into two chambers by inserting an insulated panel in the middle (U of M report: Fig. 3.1, Page 30). A 1500 W electrical heater delivered heat into the plenum through a duct dispersing the heat into the arena. The behavior of bed bugs placed in a petri-dish was studies with rise of temperature.

Results: Threshold air temperatures causing escape were above the lethal threshold temperatures (U of M report: Table 3.1, Page 33).

Conclusion: In conduction scenario, bed bugs stayed longer in harborage and tended to return to harborage as air temperatures rose in the peripheral areas. About 37.5% of bed bugs escaped from harborage to peripheral areas highlighting the need for managing airflow around the perimeter and moving stuff away from the walls.

4. *Objective 4 – Determine the rate of penetration of lethal heat through mattresses, upholstered furniture, exterior walls and other structural elements in two habitat types (apartment, house):*

Set up and variables: These were the commercial heat treatments to control bed bug populations in a single bedroom apartment and in a larger multi-level house. Direct-fired propane-fueled heaters were used for both the treatments. Temperature profiles were monitored using wireless temperature sensors as well as HOBO data loggers by placing them in general space (difficult to reach areas), between sofa cushions, mattresses, piles of clothes, closets etc. Using data loggers, temperatures were scanned every 30-60 seconds and the wireless temperature sensors reported temperature every five minutes. The data presented in the report is based on the data loggers that scanned and logged temperatures every 30-60 seconds.

Results: The rate of temperature increase was slower for the single multi-level house (2.8 to 6.5 ^{o}C/hr) compared to a single bedroom apartment (4.6 to 13.2 ^{o}C/ hr). For apartment, the bagged pile of clothes took longest to come up to the lethal temperatures. With exception of pile of clothes, the rate of temperature increase was lowest for baseboard and under the baseboard for apartment and house, respectively (U of M report: Tables 4.1 and 4.2, Page 37). Charts in the U of M report (Figs. 4.1, 4.2, 4.3, 4.4 show the rate of increase for different household articles.

Conclusion: The commercial treatment brought forth the importance of managing airflow in the space being heat treated. Achieving lethal temperatures of 48°C and above in the air space does not result in 100% kill but requires the entire thermal mass consisting of all the articles and structural members to come up to the lethal temperature. It is important to manage the airflow around the walls/periphery and also make sure that pile of clothing or any articles allow the air movement through them so that cold pockets are avoided thus eliminating areas of potential harborage for the bed bugs.

F) EXTENSION AND OUTREACH:

Dr. Kells is in the process of writing papers for scientific refereed journals like Journal of Economic Entomology or other relevant journals. He also plans on writing articles in magazines like the PCT (Pest Control Technology) as part of extension and outreach efforts.

G) DISCUSSION

The entire project was pre-dominantly research oriented and aimed at investigations that will lead to increased number of heat treatments as a safe, effective and viable alternative to the use of conventional chemical pesticides. The project definitely laid foundation for successful heat treatment with its findings on bed bug behavior and heat transfer through different material in an enclosure. As mentioned earlier, this project investigated two aspects of controlling bed bug populations:

1. *Fundamental research*:

The main reason for this project was to generate dataset on behavior and mortality of life stages of bed bugs at different temperatures and exposure times. To the best of my knowledge, no temperatures versus time mortality studies are available in the literature. It is essential to have this relationship for effective heat treatment to control bed bug infestations.

This project has yielded very valuable data on bed bug behavior and mortality which will be instrumental in performing heat treatments. The study clearly shows that a minimum temperature of 48°C is required for a minimum of 90 min for complete kill of bed bugs and also 100% mortality of eggs. Typically, eggs of insects are most difficult life stage to kill and hence achieving 48°C is important. A distinct advantage of heat is that unlike the chemical treatments, heat can penetrate cracks and crevices and inaccessible areas where bed bugs reside or harbor.

There have been reports of lower temperatures (40 to 45°C) effective in killing the bed bugs. This research proves that higher temperature (48°C) is required to ensure not only complete adult mortality but also the eggs to prevent next generation of bed bugs coming up. The research also yielded important information on behavior of bed bugs that they tend to come back to areas of higher temperatures before it gets too hot or lethal! The importance of heat treating peripheral areas and isolating the clutter from the walls was also brought forth in the research. All this information is very valuable in performing an effective heat treatment.

2. *Applied research / Commercial scale trials*:

The commercial scale trials in a single bedroom apartment and multi-level house effectively *validated* the finding of laboratory research. Additionally, these trials yielded valuable information rate of temperature increase in various articles in an enclosure. Management of air flow is key to the successful heat treatment. It is important to direct/manage airflow using network of fans and/or ductwork (high temperature fabric ductwork) so that hot air can penetrate cracks and crevices and the clutter in an enclosure. The aim here is to have lethal temperature throughout the heated space. Of course, monitoring the temperatures is critically important so that fans/ductwork can be repositioned or discharge temperature adjusted to achieve lethal temperatures.

H) HOW WILL THE PROJECT PROMOTE PROPANE USE?

Residences, apartments, low income housing groups, hospitality industry (motels/hotels), property management groups and public spaces like the , hospitals, university dorms, schools have all been battling the bed bug menace. The logistics of placing direct-fired propane-fueled (or for that matter natural gas) heaters at these establishments can be cumbersome and tedious. In high rise apartments, placement of direct-fired heaters is many times not a possibility and fuel lines going to high rise apartments or buildings can be a risky and hazardous issue. Also in spaces where use of propane-fueled heaters is possible (for example dormitories), there is a level of expertise, training and knowledge required by the service provider.

The only plausible alternative would using electric heaters in the spaces to be heat treated. Use of electric heaters to reach lethal temperatures (> 48°C) brought up issue of power requirement for the heaters and availability of outlets of adequate amperage for heaters in the rooms being heat treated. Temp Air overcame this issue by developing a mobile heat treatment system on a trailer.

1. **Temp Air's Mobile Bed bug System (EBB):**

Temp Air has developed a electric bedbug (EBB) mobile trailer system (package, specifications and illustration as Appendix 2) with an on-board 40 KW diesel generator, four heaters (7 KW each), array of fans, accessories, and a wireless real-time temperature monitoring system.

The trailer can be towed to a site such as high rise apartment or hotel/motel building and only the electrical cables from trailer are extended into the room or apartment through windows/doorways/stairwell.

The system has been very well received in the market by the local pest control companies, property management groups, hospitality industry (motels/hotels), and the Universities.

After our interaction with our existing and potential clients, we feel that there is a strong case for developing a trailer system using propane generator on-board along with a fuel tank. This system will have number of advantages as explained in the following section.

2. **Development and testing of Mobile System using Propane (PHASE 2):**

In one of our recently concluded project funded by PERC in collaboration with Purdue University, we developed a mobile heat treatment system for heat treating bins and silos (Appendix C). On similar lines, we propose to develop and test a mobile system incorporating on-board propane generator and a 150 gallon tank. I am working on developing a comprehensive proposal that will be submitted either as part of the USDA grants under the PMAP (Pest Management Alternative Programs) or EPA. Alternatively, we would like to submit the proposal to PERC for funding. Some of the issues that will be addressed are:

- i) Eco-friendly and ―Greener" alternative: Unlike the existing diesel fired system, the propane fueled system will be more environmentally friendly as propane is a clean burning fuel compared to diesel. Diesel emissions are known to be carcinogenic.
- ii) Economics: Propane is more affordable than diesel.
- iii) Start-up problems: Diesel based system has start up problem during winter. We propose a system that will utilize heat from the engine to vaporize the propane and our engineering development team informs me that start-up issues associated with diesel generator will be eliminated by use of propane generator.

iv) Need to refill and time of treatment: Present system with diesel can run for up to 8 hours and needs to refilled on a daily basis for continuous treatment. Whereas, with an on-board 150 gallon propane tank, the system can run for up to 35-40 hours making it possible to heat treat houses/apartments in far flung areas. Initial discussions with Propane utility companies reveal that they will be willing to refill the tank by driving to the site.

v) Scientific investigations: These will focus around rate of temperature increase, extensive monitoring and analysis of temperature profiles in various settings. It is also proposed to place insect bioassays in different settings to validate the laboratory results. The U of M report also highlights some of the areas that need to be further investigated for effective heat treatment.

vi) Cost-benefit and economic viability assessment

vii) Extension and Outreach

Our initial discussions on proposed propane based electric bed bug system (EBB) with local pest control companies and property management groups were very encouraging. They were willing to participate in the trials and liked the overall eco-friendly approach of the proposed system. We think that the proposed Phase 2 will result into increased use of propane as users adopt a more environmentally friendly technology. Dr. Stephen Kells has also commented on using Propane-fueled heat sources to effectively control bed bugs by heat treatment (U of M report: Pages 46-47)

APPENDIX A

More bed bug research: Stephen Kells at the University of Minnesota

Richard Shin reports for The Pioneer Press on research being done by Stephen Kells on bed bugs at the University of Minnesota.

Kells is at least one of the entomologists working on making an effective bed bug trap. Alas, ―we‗re quite a distance off" from a monitoring trap, Kells said. Eventually, it will be a wonderful invention.
It was interesting to hear how Kells got into bed bug research:
Kells first encountered a bedbug in about 2000 while working in the pest-control industry in Canada.
He dipped it into insecticide. The beast lived for four days and laid eggs.
"At that point, I knew we were in trouble," he said. *(emphasis added)*
Kells decided to study the insects further in an academic setting. He came to the University of Minnesota 3 1/2 years ago and set up a lab devoted to bedbug research. About 2,000 bedbugs live there, housed in jars, where they crawl around pieces of filter paper that vibrate and twitch with their constant motion.
They eat Red Cross-donated human blood that‗s beyond the expiration date, heated to body temperature.
Kells built a special platform he calls a bedbug arena, where he can observe the behavior of individuals when exposed to stimuli like heat. ***Part of his research is funded by the Propane Education Research Council, which wants to know whether propane-heaters can be used to kill the insects.*** *(emphasis added)*
Another set of experiments involves attaching bedbug antennae to tiny electrical probes to see what kind of chemical compounds the antennae are tuned to receive. This might help develop the lure — maybe the carbon dioxide that sleeping humans exhale or the fatty acids on our skin — for a bedbug monitoring trap.
I am trying to envision the little tiny electrodes on the little tiny antennae. I also was fascinated by the bed bugs eating expired Red Cross blood, since all the other times we‗ve read about researchers feeding their own bed bug colonies (as Lou Sorkin does) or having their grad students do it. I suppose it would be difficult to support 2,000 bed bugs.
The article also talks about the differences between captive bed bug colonies, and ―wild" bed bugs, and it cites Harold Harlan, former Army entomologist (and author of the Armed Forces bed bug guide), as the source of Kells‗ colony:
Bedbugs are a lot more resistant to poisons than they used to be. **It takes 1,200 times the amount of insecticide to kill recently captured bedbugs than it takes to kill individuals from bedbug colonies that have been in captivity for more than 30 years,** Kells said. (Entire text on the following link)

(*Source:* http://bedbugger.com/2008/04/03/more-bed-bug-research-stephen-kells-at-the-university-of-minnesota/)

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Hitch: Towing Only	**Modulating Burner**
Propane: 150 gallons; 8 hrs	**30 kW Generator Capacity**
Heater: 1.5 Million BTU's	**(4) 115 Volt Service Outlets**
Blower: 3750-7500 CFM	**Safety Switches on discharge doors**

APPLICATIONS

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Optimizing Delivery of Heat and Addressing Insect Behavior to Ensure Maximum Efficacy

FINAL REPORT

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March 17, 2009

Final Report

Applying Heat from a Propane-Supplied Heater to Control Bed Bugs In Urban and Commercial Structures

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March 17, 2009

Introduction

The common bed bug, *Cimex lectularius* L., has maintained a close association with humans and other warm-blooded animals since ancient times (Usinger 1966). The evolution of this relationship has led to the development of unique adaptations in the biology of this ectoparasite that have allowed it to exploit humans and their habitations. For example, aggregation within human nesting sites in host-adjacent harborages such as cracks and crevices of beds, furniture, floors, walls, and nearby clutter (Hwang *et al*. 2005; Potter *et al*. 2006). By locationally concealing its presence in this manner, a small reservoir population of bed bugs can increase in number within a relatively short period and begin spreading to neighboring areas, or moving to off-site locations via indirect transport on clothing, luggage, and other personal items (Kells 2006). Such adaptive behavior creates substantial problems when considering control mechanisms because of the extreme variety in and structures items requiring treatment.

Recently, bed bug infestations have become increasingly common in the United States and in other locations where they have been otherwise considered to be rare or extirpated (Paul and Bates 2000; Doggett *et al*. 2004; Hwang *et al*. 2005). Several factors have contributed to the resurgence of this pest. The increase in international trade and travel, the exchange of previously used items such as furniture and clothing, the increased frequency of residence turnover, and the evolution of resistance to residual insecticides due to repeated usage have all added significantly to the reemergence of bed bugs as a serious pest and threat to public health (Hwang *et al*. 2005; Potter 2006). Although bed bugs have never been shown to transmit disease through their bite, repeated feeding on the same host may manifest itself as itchy, lesions and can promote discomfort, anxiety, sleeplessness, and a reduced quality of life (Hwang *et al*. 2005; Reinhardt and Siva-Jothy 2007). The continued spread of this insect, and the problems caused, require control techniques that can be used in both transitional and permanent human residences.

Bed bug infestations can be difficult to manage, often times requiring ongoing and expensive inspections and treatments (Romero *et al*. 2007). Moreover, the use of conventional chemical insecticides has been met with reluctance over safety concerns and risk of resistance development. As an alternative approach, the use of non-chemical control measures such as those employing high temperatures have demonstrated practical results. Applications of high temperatures for control of insect pests in food-processing facilities has become commonplace, replacing other mass control techniques such as insecticidal fogging and fumigation. Heat treatment of structures offers another relatively safe control mechanism to which bed bugs cannot easily acquire a biochemical resistance.

When considering the application of heat treatments in food-processing facilities, and comparing its application in dwellings, there are potential limitations to heat treatments that require further investigation. One limitation may include the penetration of heat into the myriad of items and places where bed bugs may harbor. Living quarters are designed with insulative qualities that are far different than industrial facilities. Heat, cold and noise insulation are an example of protective elements used differently in living versus industrial space. Another potential limitation is the ability to survive high temperatures via physiological and behavioral mechanisms that were only rudimentarily studied in bed bugs. Overcoming these gaps in knowledge is important to ensuring that high temperature applications for bed bug control are both effective and energy efficient.

The thermal death point of bed bugs has previously reported to be 45ºC for eggs and 1°C lower for adults for a 1-h exposure (Mellanby, 1939). Mellanby (1939) reported that a 24-h exposure at 40°C is sufficient to kill all life stages. In flour mills, extensive studies are available for different food processing pests and their response to heat (Roesli et al 2003). However, the rate of heating used by Melanby is not equivalent to the application of heat in a practical control situation as demonstrated in food processing plants. The current target temperature to be reached is in a range of 47 to 52 ^{o}C, but practically there may be areas above and below these values. Typically, target temperatures are achieved by gradually raising temperatures to protect the structure and contents from over-heating. While thermal death point is important, for areas below this point, time of exposure becomes an important measure. Currently, such measures of both thermal death point and time of exposure are unknown, relative to the application of heat in a control situation.

This project will answer several important questions relative to delivery of heat into structures for the purposes of controlling bed bugs. First, while the minimum critical temperature is known (113^{o}F, Usinger 1966), little is known about lethal (or sub-lethal) temperatures with a time component incorporated. Although 120 ^{o}F will be the target temperature (at present), we need to determine if areas heated to a sub-target temperature will reach suitable conditions of temperature and time to produce the desired lethality. Second, knowing how bugs will respond to increasing temperatures will determine the rate at which items within a structure must be heated to avoid escape. Being able to move at approximately 1 m / min (Usinger 1966), it is unknown if bed bugs have the capacity to escape a zone of elevated temperatures. There are two ways that bugs may be exposed to heat, including conductive or convective heating and their response to each may be critical for determining the chances of escape. Finally, as there may be variations in structures, conducting trials during actual heat treatments will reveal if additional work is required to ensure all areas receive thorough heating.

The following objectives were completed during this project to begin filling in the aforementioned gaps in knowledge:

5. **Determine lethal temperature / time for control of bed bugs.**
6. **Determine threshold behavior and subsequent movement of bed bugs in response to increasing temperatures delivered through conduction.**
7. **Determine threshold behavior and subsequent movement of bed bugs in response to increasing temperatures delivered through convection.**
8. **Determine rate of temperature increase required for different substrates and habitat types (apartment and house) to rapidly overcome escape behavior.**

1. Determine lethal temperature / time for control of bed bugs.

Objective

The objective of the following experiments explore the limitations of heat in terms of physiological response to the thermal stress that would be experienced during a typical heat treatment as bed bugs are subjected to higher temperatures. More specifically, we examine the upper lethal temperature and lethal time of thermal exposure required to kill the egg and adult life stages of bed bugs.

Methods

Live bed bugs were collected from six commercial properties located in Minnesota, Wisconsin, Florida, and New Jersey and combined to form the ECL‘06 colony in 2005 (Olson et al. Pers. Comm.). Bed bugs from this field colony were cultured in 473 mL glass jars containing several 9.0 cm dia filter papers for harborage and covered with fine mesh for ventilation. The colony was maintained at 27°C and 70%RH under a 16:8 L:D photoperiod in a controlled environment chamber (Percival Scientific, Inc.; Perry IA). Bed bugs were fed whole, heparinized human blood through an artificial membrane system similar to Montes, *et al*., 2002. Human blood was procured from expired stocks purchased from the American Red Cross (St. Paul, MN).

For experiments using adults, 10 fed or 10 unfed adult bed bugs per vial were placed in 6 mL plastic sample vials containing a 1 cm x 3 cm filter paper strip for harborage. Bed bugs categorized as —fed” were provided blood *ad libitum* and used in experiments within 24h of feeding. Bed bugs that were categorized as —unfed” had not been offered a blood meal at least 14 days prior to use in experiments. A screw cap with a 4mm hole, blocked with filter paper, allowed for ventilation. Vials containing adult bed bugs were maintained under conditions identical to the field colony until use.

For egg collection, at least 10 engorged adult bed bugs per vial were placed into 6mL plastic sample vials containing several 1cm x 3cm filter paper strips for harborage, as well as to offer a substrate for oviposition. A screw cap with a 4mm hole, blocked with filter paper, allowed for ventilation. Sex ratios in the vials were heavily weighted in favor of females. Vials were maintained under conditions identical those of the field colony. After 5 days, viable eggs were collected from the filter paper strips and immediately used in experiments. At least 10 eggs per vial were placed with their substrate into 6mL plastic sample vials identical to those mentioned above.

Two heating devices were used for exposing bed bugs to elevated temperatures. First, a controlled environment chamber was programmed to increase the temperature at a constant rate of 0.06°C/min or 3.6°C/h from 23°C up to a maximum of 50°C. Temperature rate was selected based on preliminary discussions with Temp-Air personnel (Hulasare, Pers. Comm, unpublished data). Due to limitations on the maximum temperature output provided by the environmental chamber, the oven of a gas chromatograph (GC) (6890N, Agilent Technologies, Inc.; Santa Clara, CA) was employed for experiments when required temperatures exceeded 50°C. Also, with a smaller volume and better heater control, the GC oven was used in experiments where time periods were short. The lowest obtainable temperature for the GC was 30°C and this temperature served as the starting point for all experiments.

Vials containing adult fed or unfed bed bugs or eggs were placed in the GC oven and the temperature was allowed to increase at a constant rate of 0.06°C/min from a starting temperature of 30°C. Vials were randomly assigned to a 10-min temperature exposure of 30, 35, 40, 43, 45, 50, and 55°C. Control vials containing adult fed or unfed bed bugs or eggs were placed in the environmental chamber and maintained at 30°C. When the GC reached one of the treatment temperatures, a vial was randomly removed from the oven along with its corresponding control vial. All vials were then held for 24 h at room temperature, after which time mortality was recorded and dead bugs were removed. All vials were then returned to conditions identical to the field colony for 2 weeks. At 1- and 2-week intervals following temperature exposures, remaining living bugs were offered a blood meal. The number that successfully fed was recorded and those bugs were removed from the vials along with any dead bugs. At the end of the 2-week interval, the number of emerged nymphs was recorded from vials originally containing eggs.

For determining time of exposure on bed bug mortality, vials containing adult fed or unfed bed bugs or eggs were placed in the environmental chamber and the temperature was allowed to increase at a constant rate of 0.06°C/min from a starting temperature of 23°C. Vials were randomly assigned to exposure times of 2, 10, 20, 40, 60, 90, and 120min at a temperature of 30, 35, 40, 43, 45, 48, 50, or 55°C. Control vials containing adult fed or unfed bed bugs or eggs were set aside at room temperature. When the appropriate exposure time and temperature were reached, the assigned vials were transferred to room temperature for 24h. After 24h, mortality was recorded and dead bugs were removed. All vials were then returned to conditions identical to the field colony for 2 weeks. At 1- and 2-week intervals following treatment, remaining living bugs were offered a blood meal. The number that successfully fed was recorded and those bugs were removed from the vials along with any dead bugs. At the end of the 2-week interval, the number of emerged nymphs was recorded from vials originally containing eggs.

For lethal temperature, data were processed through logistical regression via Proc Probit (SAS 2004) with Probit (mortality) versus temperature at which the bed bugs were removed. This enabled calculation of lethal temperature estimates with 95% fiduciary confidence intervals at 50 and 99% mortality. Eggs and adults could then be compared based on the slope response and LTemp50, 99 values. For lethal time, data were first graphed to determine the presence of sigmoidal relationships similar to lethal temperature, but analysis used evaluated mortality versus time of exposure. Temperatures where there was < 100% mortality of bed bugs after 12 hours were considered as not effective for a heat treatment. Temperatures where all bed bugs were dead at the time when the experiment started were considered above the threshold thermal death point. The temperature where a sigmoidal curve existed was considered the threshold for when mortality would be initiated.

Results

Effects of lethal temperature on survival and emergence

There was no significant difference in high-temperature survival between fed and unfed bed bugs, as evidenced by the 95% fCI overlapping both LT50,99 estimators and non-significance between the slopes. As a result the data were combined into one analysis. Also, mortality of control bed bugs at 30^{o}C was negligible at, or below, 10% during these experiments. Initiation of mortality occurred over a narrow range starting at 40 ^{o}C, with 100% mortality at 50 and 55 ^{o}C (Figure 1.1). Bioassay analysis yielded estimates of LT50 43.1 (40.5, 45.0) ^{o}C and LT99 48.11 (45.8, 64.6) for population mortality. Failed emergence of eggs two weeks post emergence yielded a similar analysis with LT50 44.6 (42.2, 46.6) ^{o}C and LT99 52.3 (49.2, 63.8).

Effects of exposure time on mortality and emergence

The effect of increasing exposure time on survival at set temperatures for adult bed bugs is shown in Figure 1.2. Data for fed and unfed bed bugs were combined because there were no differences in nutritional status. Mortality did not exceed 25% for temperatures less than 45°C at all exposure times. There was an increasing trend towards greater mortality of adult bed bugs starting at the 20-min exposure to 45°C that culminated in complete mortality at 90-min of exposure. At 60-min exposure, approximately 50% of adult bed bugs exposed to 45°C did not survive. Complete mortality was obtained at temperatures 48, 50, and 55°C for all exposure times except 10-min at 48°C (90%).

The effect of increasing exposure time on emergence of nymphs at increasing temperatures is shown in Figure 1.3. Emergence of nymphs was consistently above 90% for temperatures below 48°C at all exposure times except 20-min at 43°C (75%) and 90-min at 45°C (83.3%). At 48°C, there was a decreasing trend of nymphal emergence. Emergence of nymphs fluctuated between 0 and 10% for exposure times starting at 20-min for 48°C. Except for 1 living nymph found after the 20-min exposure to 48°C, all

Figure 1.1. Mortality of bed bugs over a range of temperatures.

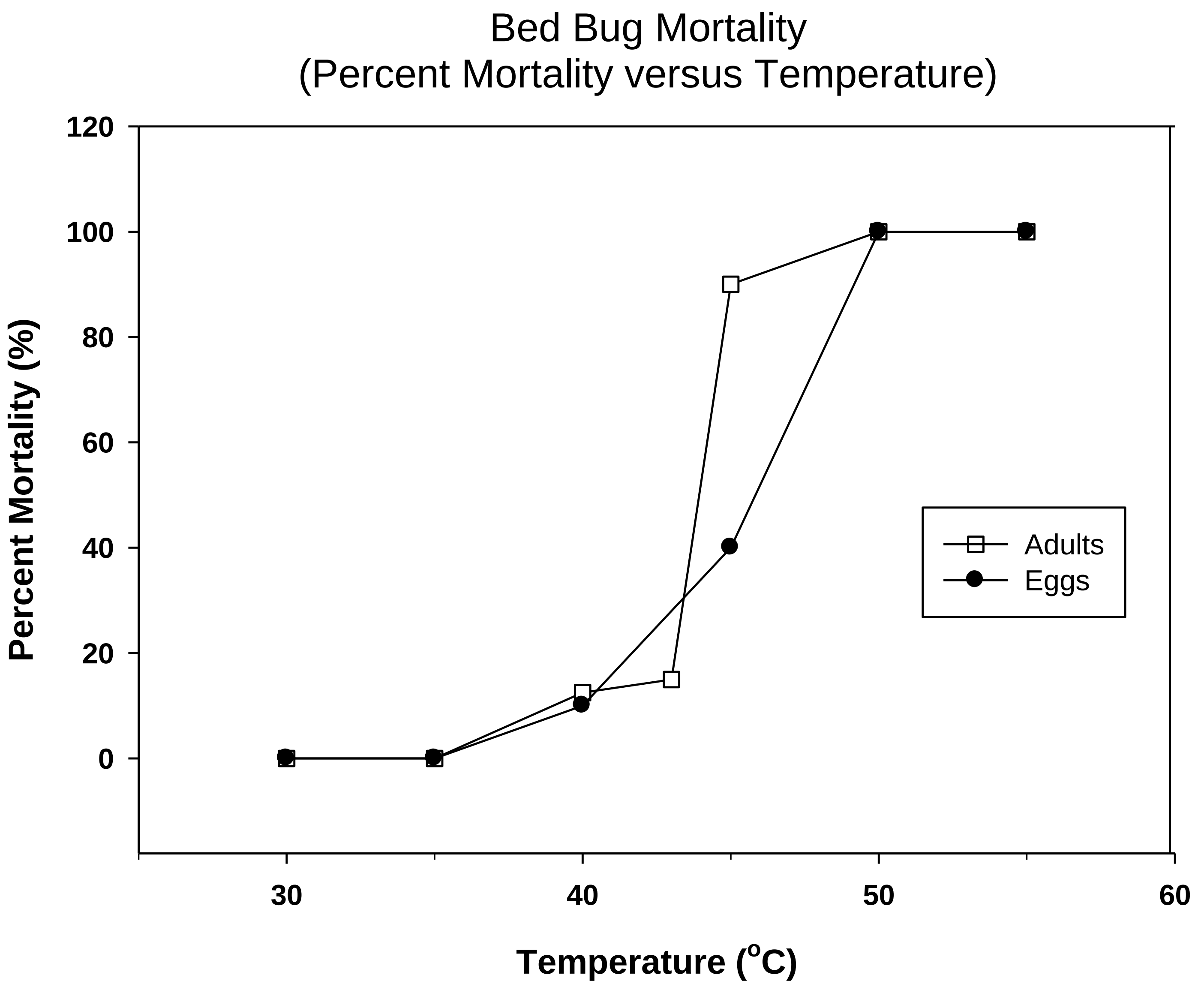


Figure 1.2. Bed bug mortality over time at set target temperatures and regardless of nutritional status.

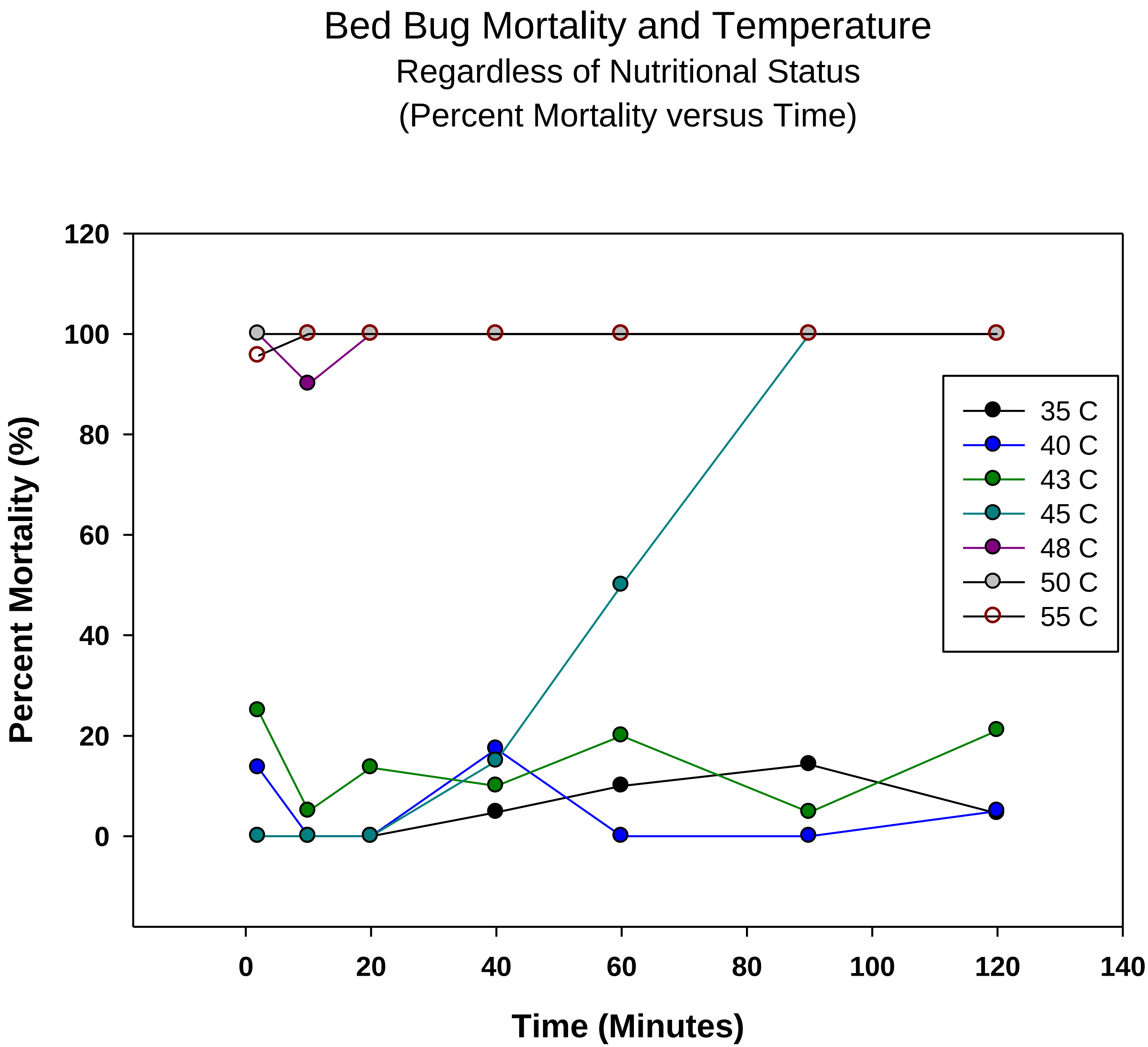


Figure 1.3. Emergence over time of bed bug eggs exposed to a range of set temperatures.

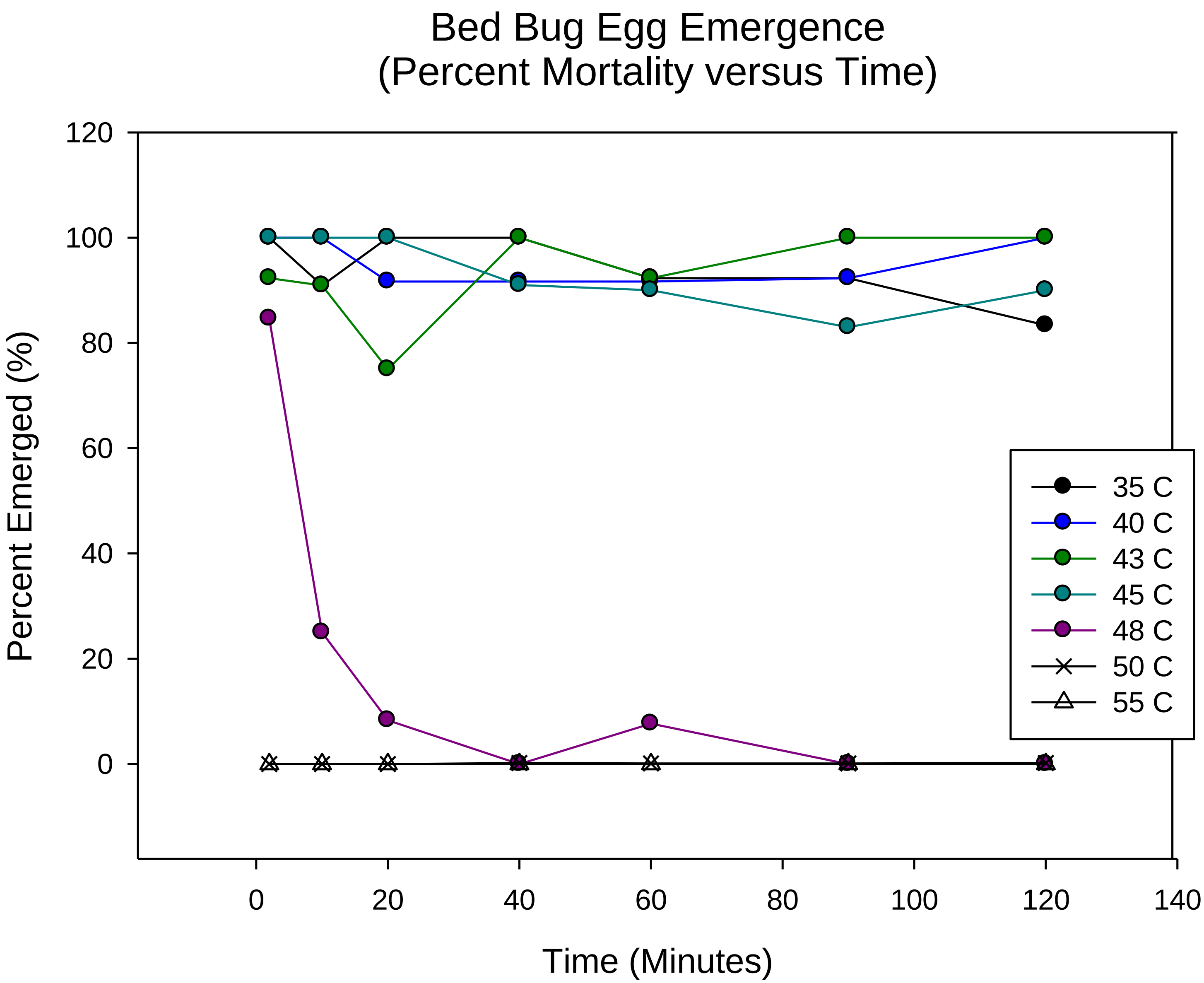


Table 1.1 Minimum exposure times for heat treatments to obtain complete mortality of bed bug eggs when heated at a rate of 3.6°C/h.

	Emergence but failed development		Complete Failed Emergence
Temperature (°C)	Exposure Time	% Emerged	
45	4 Hours	20 %	8 Hours
48	40 Minutes	8 %	90 Minutes
50	--	--	0 Minutes

other nymphs that had emerged were subsequently dead. There was no emergence of nymphs at 50 and 55°C for all exposure times (Table 1.1).

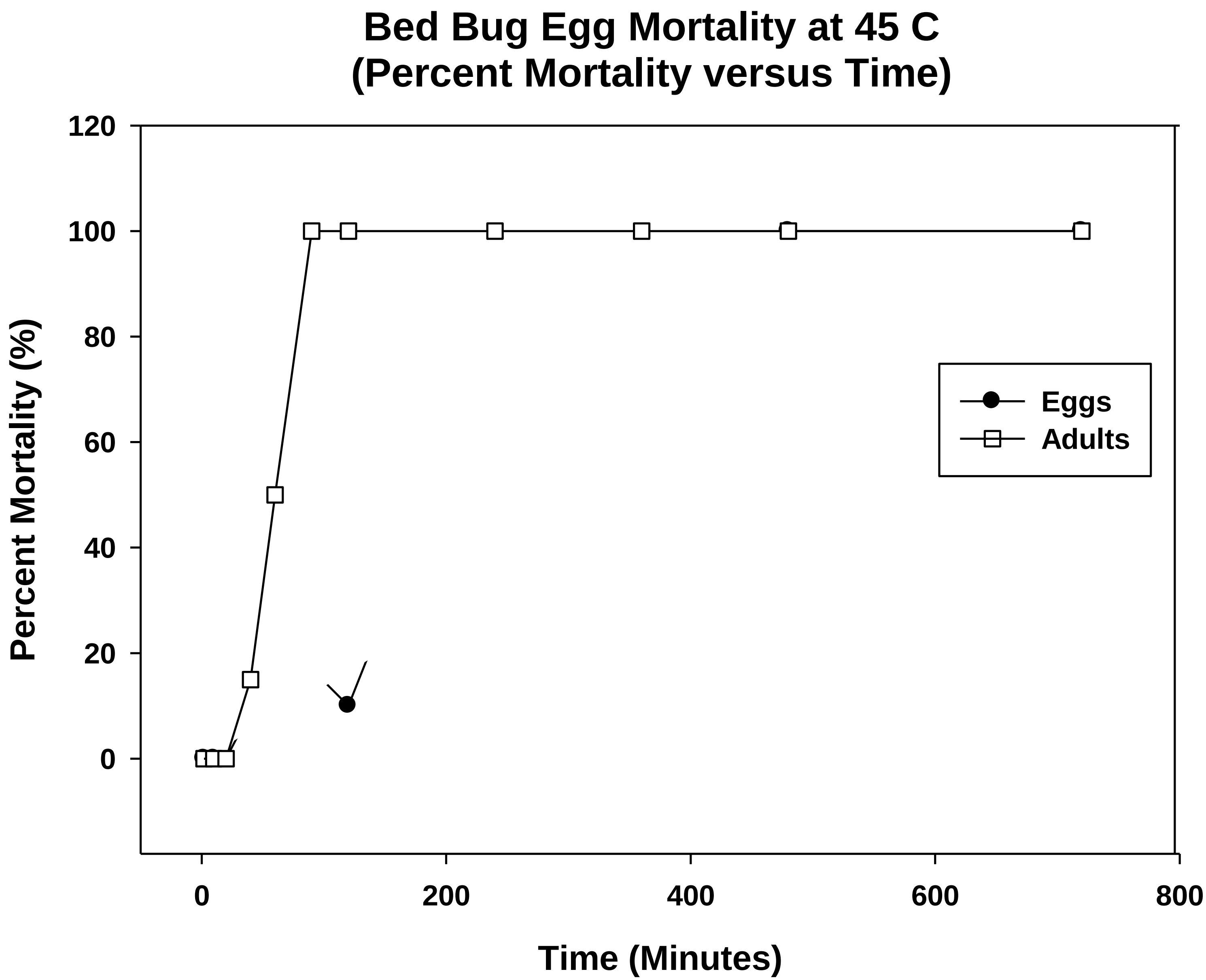
Effects of extended exposure time at 45°C on mortality and emergence

To study the effects of extended exposures at 45°C, adult bed bugs and eggs were exposed to 4, 6, 8, and 12h in addition to previous time points. Mortality of adult bed bugs was substantially different from emergence failure of eggs at extended exposure times at 45°C (Figure 1.4). Complete mortality of adult bed bugs was recorded at exposure times at and beyond 2h. The expected lethal time at 50 (LT_{50}) and 99% (LT_{99}) were 58.0 and 94.8min, respectively. Eggs were far less sensitive to increasing durations of 45°C exposure than adults. Emergence failures were less than 20% for exposure times between 2 and 120 min, after which failure to emerge rose sharply. Levels of emergence failure were 80 and 90% for 4 and 6h exposures, respectively. Furthermore, those nymphs that had emerged at the 4 and 6h exposures did not survive (Table 1.1). Complete emergence failure was recorded at and beyond 8h. Simple linear regression estimates that the LT_{50} = 194.1min for eggs.

Conclusion

Our study indicates that bed bugs may survive elevated temperatures when the temperature is slowly increased. These temperature ratings are different from previously recorded values (Usinger 1966) and those reported at the recent 2009 ESA meeting. One possible explanation for the disparity is the method of heating and the rate at which bed bugs experience high temperatures. Often evaluation of high temperature survival is accomplished by immersing the insects into a high temperature environment – usually a

Figure 1.4. Mortality over time for bed bug adults and eggs exposed to 45 ^oC.



water bath or similar. It is uncertain what specific mechanisms are available for the bed bugs to withstand these higher temperatures and the requirements to stimulate induction of these mechanisms. It may be a result of heat shock proteins, similar to other insects (Guedes et al. 2008 and others). Further study in a side-by-side experiment (slow heat up versus fast heat up) will be necessary to determine if there is a mechanism existing for resisting heat stress. Overall, the temperature requirement to control bed bugs is within reasonable parameters, though higher than originally expected.

2. Determine threshold behavior and subsequent movement of bed bugs in response to increasing temperatures delivered through conduction.

Objective

The objective of the following experiment was to expose bed bugs to a surface area of heat delivered through conduction and determine how they move relative to increasing temperatures. This method of heating would occur as heat is transferred to walls and into items where the insulative qualities prevent free air movement.

Methods

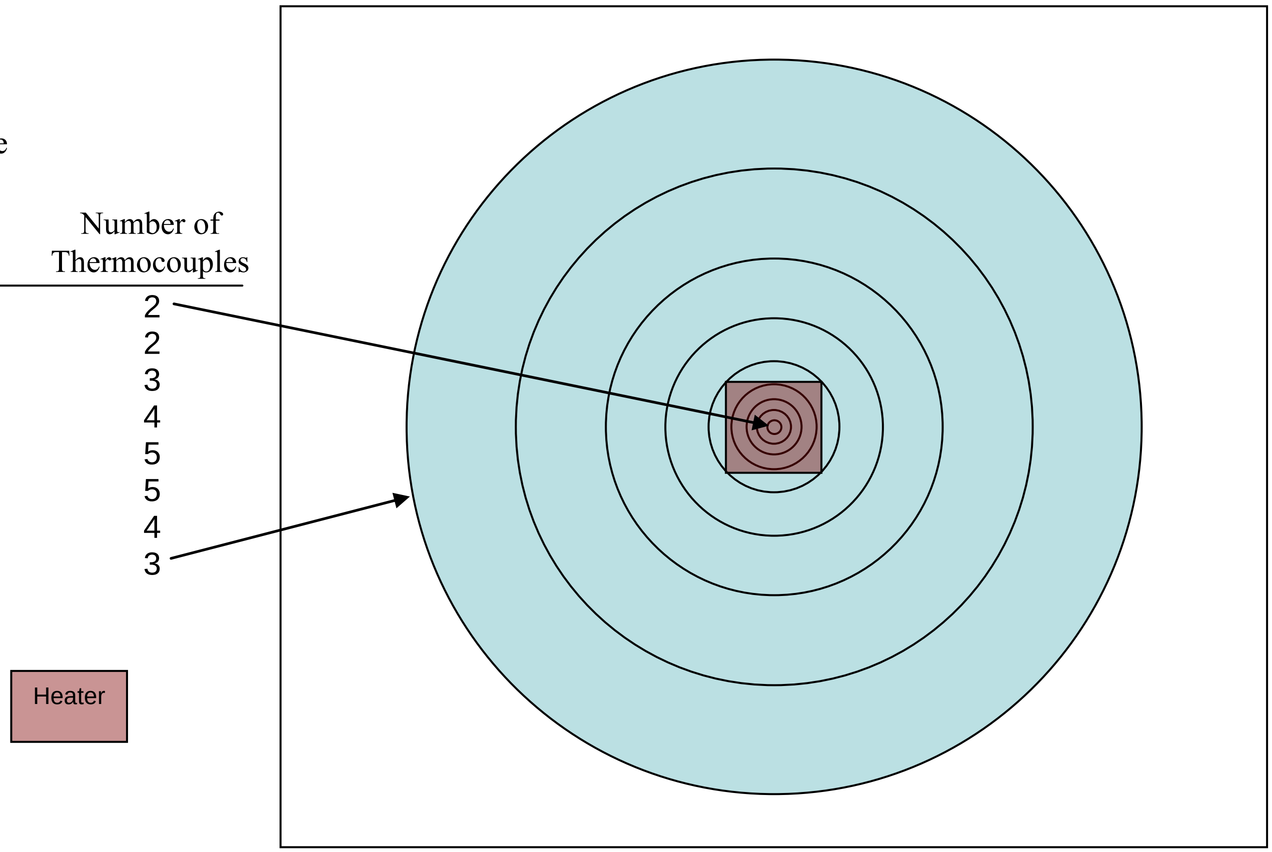
Bed bugs were observed in an arena consisting of a heater placed in contact with a 1 m^2 x 3 mm aluminum plate. Surrounding the heater and supporting the aluminum was Styrofoam® insulation, which focused heat dissipation across the aluminum plate. Thermocouples were placed in concentric circles radiating out from the center of the heater to monitor the development of temperature across the aluminum plate (Figure 2.1). These thermocouples were set to scan temperatures at 20 second intervals. A linen sheet stretched over the aluminum provided a surface for bed bug movement. In addition to the thermocouples, a non contact (infrared) thermometer was employed to monitor surface temperatures. This assembly was constructed after many trials with different materials. The aluminum plate replaced wood materials because there was enough insulative resistance with wood products resulting in a very small lateral heat gradient.

The arena was completed by a 10 cm high plexiglass wall. In the center of the arena a piece of harborage consisting of soiled filter paper (1 cm diameter) provided a

Figure 2.1. Schematic of conduction arena and the number of thermocouples placed around each radius.

Schematic of Thermocouple Placement

Distance from center (cm)	Number of Thermocouples
1	2
1.7	2
2.7	3
4.5	4
7.5	5
12.3	5
20.3	4
33.5	3



starting point for the bugs. A plexi-glass lid (approx $1m^2$) was placed on top of the bordering walls. In the center of this lid, a 2.5cm hole permitted removal of a containment cylinder which released the bed bug, starting the experiment.

During preliminary trials, we determined a means to estimate surface temperature without interfering with bed bug movement. Changes in movement by bed bugs could occur as a result of interfering with light coming from above the arena, the release of CO_2 into the arena, or aberrant air currents. Surface temperatures were calculated based on the thermocouples placed under the arena. In the preliminary trials (n=5), pairwise recordings of both sub-surface (thermocouple) and surface (infrared) temperatures were recorded during a 15 minute heating phase. Surface temperature was then matched with the corresponding sub-surface thermocouple reading. The temperature lost between subsurface and surface was expressed as a ratio:

$$T_{Lost} = T_{IR}/T_{TC}$$

where T_{IR} is the infrared temperature and T_{TC} is the thermocouple temperature. A regression of T_{Lost} versus T_{TC} determined an expression that would provide a surface temperature estimate for each T_{TC} measured with bed bug behavior.

To initiate the experiment, a bed bug was placed inside the containment cylinder with the harborage. The cylinder was blocked with a piece of paper and placed in the center of the arena. Once in place, the paper was slid out from between the bed bug and the arena. With the bed bug visibility calm, on or under the harborage, the containment cylinder was lifted. Bed bugs that immediately moved were replaced within the containment cylinder. Bed bugs that continued to remain at the harborage site permitted the experiment to begin.

The observation period was started by turning on the ceramic heater and triggering the pre-armed datalogger. During the experiment, behavioral changes in the bed bug were observed and recorded along with the time (minute:second) that this behavior occurred. Bed bug movement was observed within the 33.5cm radius and when bed bugs exited this radius, the observation continued until a total of 10 minutes had elapsed. In some cases, observations may have exceeded 10 minutes if the bed bug was still within 33.5cm from the center (though they may have exited and then re-entered the 33.5 cm radius).

Following completion of the experiments, behavioral events (i.e., any time the bed bug exhibited a change in behavior) for each bed bug was summarized with the time, distance from the center, and the average temperature from the thermocouples at the nearby radius. Distance moved or spatial locations of the bed bug were ascertained by a series of concentric lines on the aluminum plate that matched thermocouple placement. These lines were visible through the linen sheet. Radius from the center may be precise if the bed bug was located directly above a radius line. However when the bed bug was in between the lines, distance was estimated in cm if the bed bugs were closer to one concentric line than the other. If the bed bugs were approximately half way between the lines, this characteristic was also noted. Estimates were necessary because visual observation of the bed bug was made at a distance > 1 m to avoid observer influences on bed bug movement. With the distance and time known, the average thermocouple temperature was assigned to the behavior observed.

Results

The surface temperature was estimated by linear regression of T_{Lost} versus the thermocouple temperature (T_{TC}). This regression provided the equation:

$$T_{Lost} = 1.1058 - 0.0055\ T_{TC}$$

with an acceptable r^2 of 0.942 (Figure 2.2). This equation was used to determine the correction factor that, when multiplied by the T_{TC}, yielded the surface temperature. The heater raised the temperature of the harborage by 5.24 ^{o}C / minute. Lethal conditions in the harborage began at 4 minutes into the arena heat up and at the conclusion of the 10 minutes the lethal zone had moved out to a radius of 8 cm (Figure 2.3)

There were three specific behavioral events that were expected during the heat up. The main expected event was escape from a lethal surface temperature. The other two main events that could be expected included a latency period before the escape event and the post-escape period. While the escape event was expected, specific behaviors during the pre- and post- escape were largely unknown. During the pre-escape period, a number of behaviors were observed including:

- Change of position – the bed bug moves its position close to the harborage. The bed bug may move on top or underneath harborage or it may move around the perimeter of the harborage.
- Movement into proximate areas. The bed bug disengages itself from the harborage and moves into neighboring regions. Often the bed bug returns to the harborage.
- Attempted feeding – Bed bugs attempt to probe the arena surface with their proboscis

Figure 2.2. The relationship between temperature loss at the arena surface versus the measured temperature under the surface. Temperature loss is expressed as a fraction (or ratio) of the surface temperature as measured by non-contact IR thermometry (T_{IR}) and the thermocouple temperature (T_{TC}) under the arena floor. The resulting regression equation is used to estimate surface temperature available to bed bugs, based on sub-floor measurement.

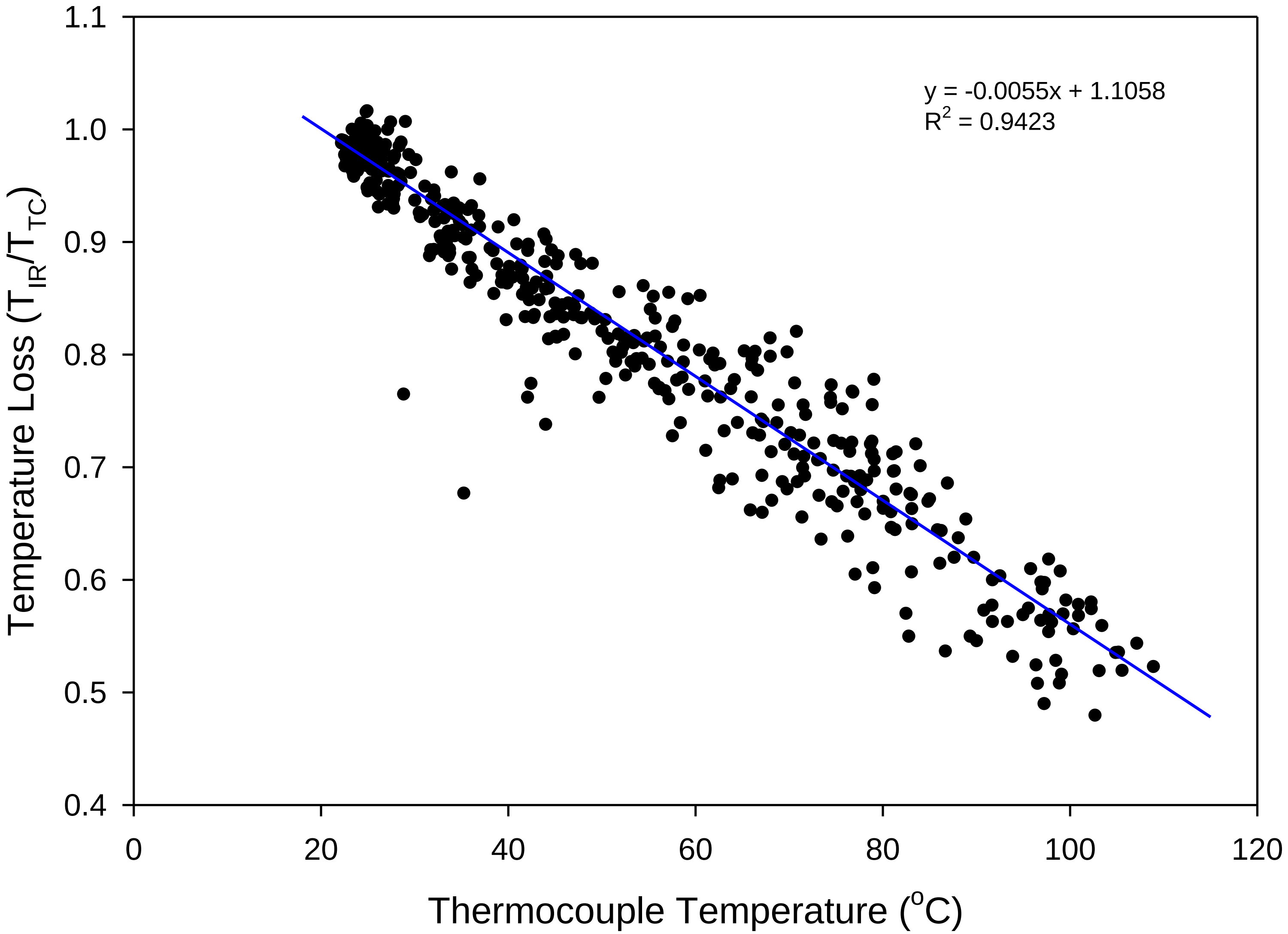
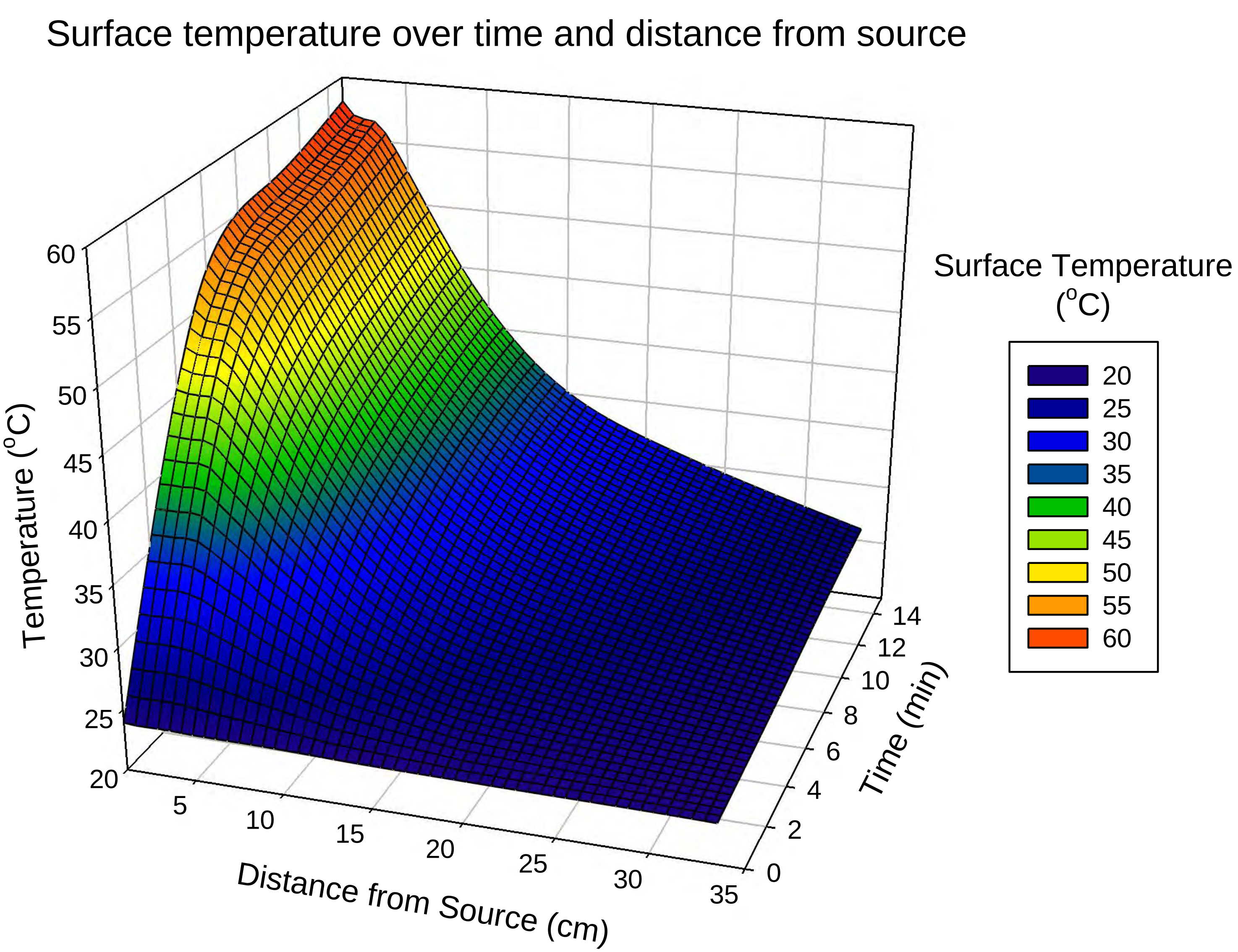


Figure 2.3. Profile of surface temperature as affected by time and distance from the heat source.



Escape behavior was characterized by agitated or increased movement, stilting (body raised above surface), a rapid movement to the top of the harborage, and a rapid movement out of the lethal zone. The post escape period include several behavioral events, such as:

- Feeding – as described above.
- Changes in position in the arena or ―looping behavior" – sometimes the bed bug moves into a cooler part of the arena, then turns and moves back toward the harborage. Upon reaching a temperature it may reside there, it may turn back to cooler areas. Looping behavior occurred when the bug repeatedly combined advancing and retreating behavior. It is uncertain if this behavior was the result of foraging, or attempt(s) to return to the harborage.
- Exiting the arena – where the bed bug leaves the 33.5 cm distance and locates along the arena's wall or in the corner. The bed bug may re-enter the arena one or multiple times until finally positioning itself next to the arena wall.

Bed bugs began their initial movements when the surface temperature reached an average of 27.2 ^{o}C (with a 95% confidence interval of 26.1, 28.3) (Table 2.1). Most of this behavior was defined as a change in position or movement into proximate areas. Feeding was initiated by starved bed bugs when the temperature reached 35.2 (31.4, 38.9) ^{o}C (Table 2.2). No fed bed bugs attempted feeding and approximately half of starved bed bugs attempted feeding (overall 29.6% of all bed bugs attempted feeding). These various behaviors occurred until the pre-escape threshold of 35.9 ^{o}C was reached that being the last temperature recorded before escape was attempted (Table 2.2).

Table 2.1. A summary of threshold temperatures where exploratory behavior first started and the highest average temperature before escape behavior occurred.

Condition	Threshold for Movement (^oC)			Pre-Escape Threshold (^oC)		
	n	Average	95% Confidence Interval	n	Average	95% Confidence Interval
Starved Female	7	26.7	(26.2, 27.2)	7	38.4	(34.6, 42.2)
Starved Male	6	27.8	(24.6, 31.0)	6	37.3	(33.1, 41.5)
Fed Female	7	28.2	(24.9, 31.4)	8	35.9	(32.8, 38.9)
Fed Male	6	26.1	(25.3, 27.0)	6	31.4	(27.6, 35.2)
Overall	**26**	**27.2**	**(26.1, 28.3)**	**27**	**35.9**	**(33.9, 37.8)**

Table 2.2. A summary of surface temperature conditions at the point when bed bugs attempted to feed.

Condition	Threshold for Feeding (^oC)			Attempted Feeding (%)
	n	Average	95% Confidence Interval	
Starved Female	3	36.8	(28.5, 45.1)	42.9
Starved Male	4	33.9	(30.8, 37.1)	62.5
Fed Female	0	--	(--, --)	0
Fed Male	0	--	(--, --)	0
Overall	**7**	**35.2**	**(31.4, 38.9)**	**29.6**

Escape behavior was initiated at temperatures of 41.0 (38.5, 43.5) °C (Table 2.3). A large portion of fed bed bugs had no escape behavior, instead they had gradual movement away from the harborage without the typical ―panicked" or excited movement. A portion of fed bed bugs (approximately 15%) were unable to escape from the lethal zone, usually a result of exiting to the top of the harborage then struggling across the hot (>50 °C) areas. Stilting behavior was not observed in fed bed bugs and a close proximity of their body to the surface may have caused a gradual departure or rapid heating of the body. Also, fed bed bugs are negatively thermo-tactic and this may have lead to the escape behavior being lower did occur, it happened at a lower temperature.

During post-escape behavior, bed bugs tended to approach surface temperatures of 30.1 (28.5, 31.5) °C (Table 2.4). This approach may be related to movement away from a higher temperature to the cooler temperature, or positive taxis from a colder surface to the warmer surface. Post feeding behavior occurred at 31.9 (28.9, 34.9) °C (Table 2.4).

Conclusions

1. Bed bugs do not immediately try to escape the heating field, many of them will actually forage or feed.
2. The difference in temperature between foraging / movement and escape is relatively close. This ensures many of the bed bugs will likely be contained.
3. Escape may occur and it is advisable to apply insecticides to perimeter areas where a gradient of temperature may occur.

4. In areas where a temperature gradient occurs, bed bugs will not simply run away. They will search in warmer areas, which may permit a lethal zone to develop around their harborage before they can escape.

Table 2.3. A summary of the average escape threshold for bed bugs attempting to move from a heated surface, the percent of bugs that demonstrated no escape behavior, and the percent that succumbed to high temperature while attempting escape.

Condition	Escape Threshold (^oC)			No Escape Behavior Observed (%)	Died While Attempting Escape (%)
	n	Average	95% Confidence Interval		
Starved Female	7	42.3	(39.9, 44.8)	0.0	0.0
Starved Male	7	41.9	(37.2, 46.7)	0.0	0.0
Fed Female	8	42.9	(36.8, 49.0)	25.0	14.3
Fed Male	6	35.8	(31.6, 40.1)	50.0	16.7
Overall	**28**	**41.0**	**(38.5, 43.5)**	**17.9**	**7.1**

Table 2.4. After escaping lethal temperatures, the majority of bed bugs reentered the arena, attempted a return to the harborage, foraged or attempted to feed in the arena. The average highest temperature selected by bed bugs during reentry and surface temperature conditions resulting in post-escape feeding are presented.

Condition	Highest Sfc Temp Encountered			Surface Temps: Post-escape feeding behavior			
	n	Average	95% Confidence Interval	n	Average	95% Confidence Interval	Attempted Feed (%)
Starved Female	7	30.7	(28.1, 33.3)	1	34.9	(--, --)	14.3
Starved Male	7	29.7	(26.8, 32.6)	2	30.4	(29.5, 31.2)	37.5
Fed Female	6	31.9	(27.7, 36.2)	0	--	(--, --)	0.0
Fed Male	4	27.2	(27.0, 27.3)	0	--	(--, --)	0.0
Overall	24	30.1	(28.5, 31.7)	3	31.9	(28.9, 34.9)	16.7

3. Determine threshold behavior and subsequent movement of bed bugs in response to increasing temperatures delivered through convection.

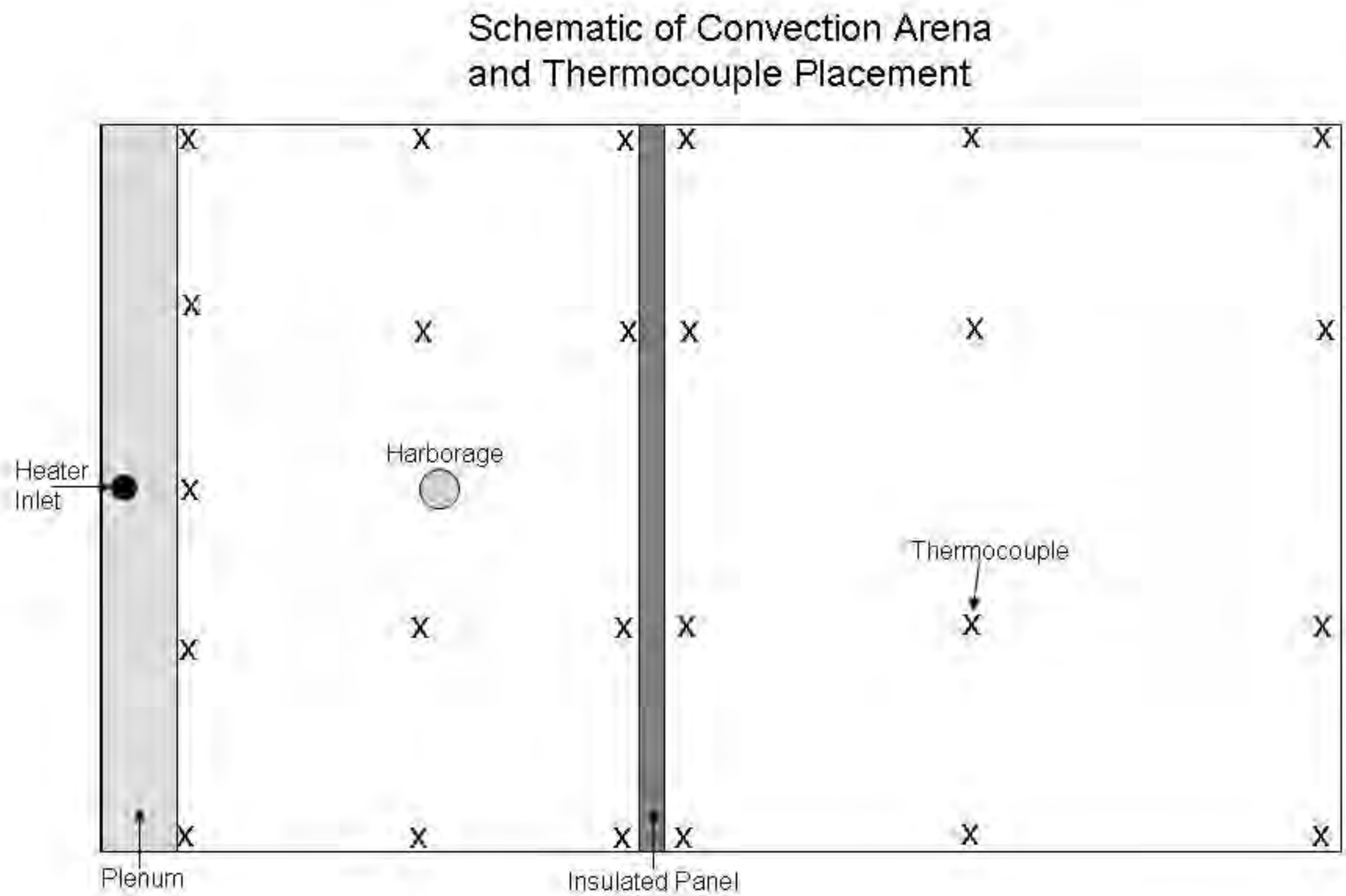
Objective

While conduction of heat is a possible route of exposure to heat, heating through convection is more likely. This part of the study exposed resting bed bugs to increasing temperatures delivered by heated air flow. Key measures of concern include the threshold temperature when bed bugs begin to move and whether bed bugs would escape the heating field.

Methods

The demonstration case used by Temp-air was modified into a two chamber arena (Figure 3.1). This case (1 m x 60cm x 20cm high) is constructed of aluminum with one side and the top lid made of Plexiglas for observing bed bug behavior. The floor of the arena consisted of plywood sealed with epoxy paint, with edges sealed to the walls with tape. Heat was delivered from a 1500 W heater through a hose (5 cm dia) and into a plenum (10cm x 60cm). The plenum dispersed hot air across the chamber floor. To divide the arena into two chambers, an insulated panel (Styrofoam® board 5cm" thick) was placed across the centerline of the arena. At the base of the panel, there was a 0.6 cm gap along the bottom which enabled heat to move into the second chamber and permitted a heat refugia for bed bugs. Preliminary tests of temperature distribution within the chamber were accomplished by placing thermocouples at specific points within each chamber. Thermocouples were placed just above the surface of the chamber, so there

Figure 3.1.Schematic of convection arena and placement of thermocouples.



was no interference of the surface temperature. The thermocouples were connected to a Personal Daq datalogger and connected by USB to a personal computer. The data logger scanned temperatures every 20s. The preliminary trials were run until temperatures stabilized and four replicates were performed.

As bed bugs can be affected by objects in the arena, the number of thermocouples were reduced to five; including one thermocouple at the harborage and 2 thermcouples on either side of the insulated panel. Bed bugs were placed in the arena similar to the heat conduction experiment with the exception that a petri plate was placed over the harborage to protect the bed bugs from a direct air flow. Bed bugs could move from under the plate because half of the rim was removed. As per the conduction experiment, both fed and unfed bugs were used.

The bed bug was placed close to a piece of soiled harborage and permitted a period to calm. Once calmed, the heater turned on and simultaneously the thermocouples were triggered to record data. Similar to the conduction experiment, bed bugs were observed for behavioral changes and these behaviors were recorded along with the time of occurrence. The experiment continued until the bed bug was dead, the bug escaped out of the heat field, or the bug remained peripheral to the heat field but outside the lethal range (up to 30 minutes).

After each experiment, the chamber was permitted to cool to room temperature and the bed bugs were removed. Behavior of each bed bug was summarized and the corresponding escape temperature was found by matching the time that the behavior occurred with the scan time from the datalogger.

Results

The temperature profile of the arena was such that most areas were above the lethal threshold for the bed bugs. The areas providing a refuge included the two corners where there was a dead air space created by the Styrofoam contacting the chamber floor (Figure 3.2). These spaces were necessary to maintaining the gap along the rest of the divider. Regardless, the lethal temperatures were apparent in most areas and in areas where it was below lethal threshold, the temperatures were above the escape thresholds encountered during the conduction experiment.

Threshold air temperatures causing escape behavior were at a level above the lethal threshold temperature (Table 3.1). There were significant differences among fed or starved individuals, but the majority respond above 45^{o}C. In this chamber, there was a greater mortality and fewer escapes from the heated area (Table 3.1). Overall, 62.5% of bed bugs died within the area, while 37.5% successfully escaped. Over half the bed bugs reentered the harborage after leaving in the attempt to escape (Table 3.1).

Conclusions

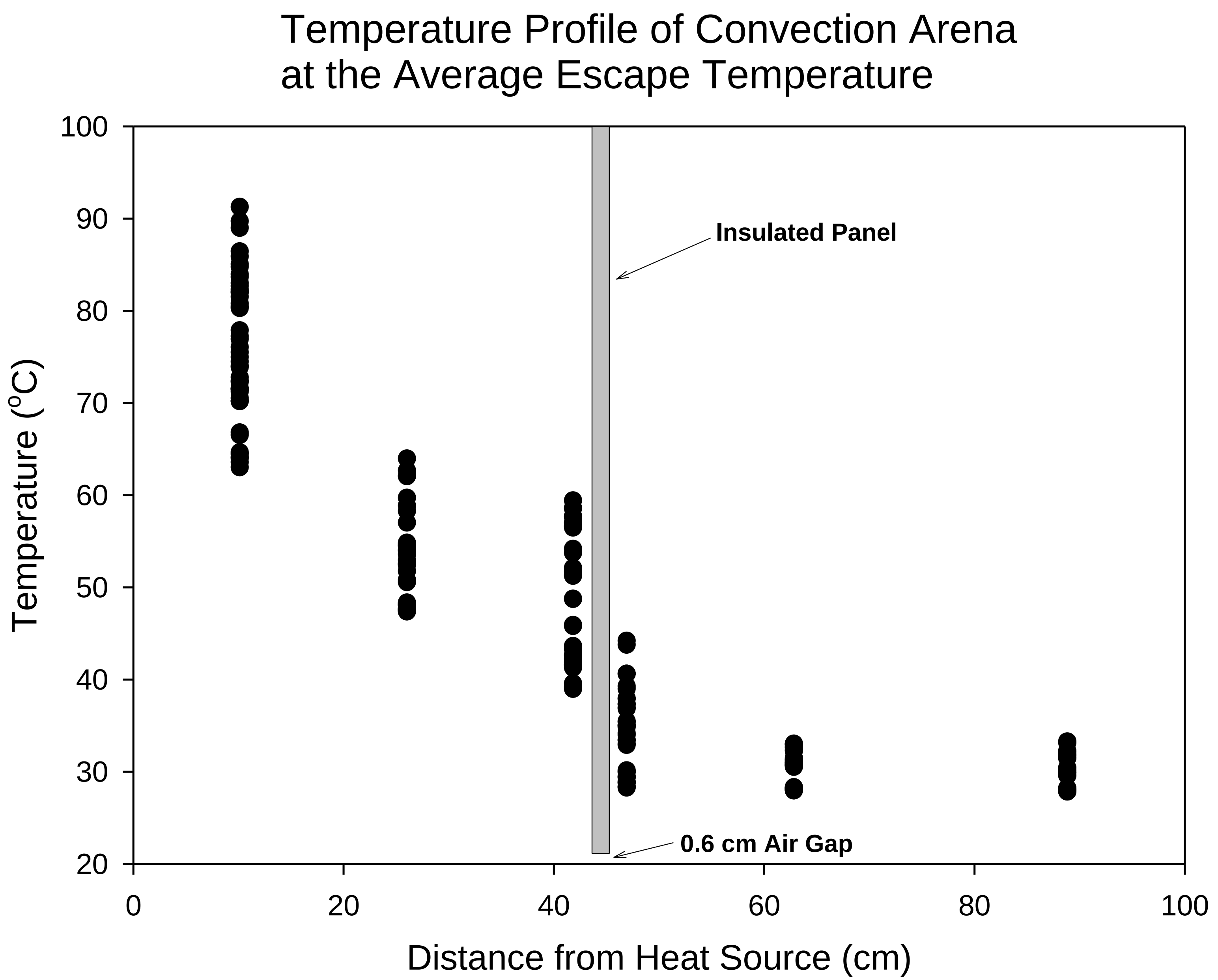
1) Bed bugs were prone to wait in harborages when heat was delivered via air. Their threshold for escape was substantially higher.
2) Bed bugs showed a stronger propensity to stay with the harborage or would return to the harborage as air temperatures began heating the peripheral area.
3) While the distances were relatively small between the heated areas and refugia only 37.5% of bugs actually escaped. Measures to prevent bed bugs from

escaping may include moving infested articles away from the walls and the use of directed air around the perimeter, before heating the interior.

Table 3.1. A summary of the average escape temperature threshold for bed bugs attempting to move from an arena with the heated air, including the percent of bugs died inside or outside the harborage, the percent that successfully escaped the heat field and the percent that attempted to re-enter the harborage.

Condition	n	Escape Temperature		Percent of individuals			
		Average	95% Confidence Interval	Dead inside Harborage	Dead outside Harborage	Escaping heat field	Attempting to Regain Harborage
Starved Female	8	48.4	(42.9, 53.8)	37.5	50.0	12.5	6.3
Starved Male	8	45.1	(41.9, 48.3)	25.0	50.0	25.0	87.5
Fed Female	8	51.9	(49.0, 54.8)	25.0	25.0	50.0	12.5
Fed Male	8	45.2	(40.5, 50.0)	0.0	37.5	62.5	50.0
Overall	8	47.6	(43.2, 52.1)	21.9	40.6	37.5	53.1

Figure 3.2. Temperatures measured in convection arena at time of the average escape temperature of 47.6 °C. The variability at each distance is a factor of replicate (n=4) and measurement of four points at each distance (across the arena).



4. Determine the rate of penetration of lethal heat through mattresses, upholstered furniture, exterior walls and other structural elements in two habitat types (apartment, house).

Objective

During a heat treatment to control bed bugs, the availability of thermal refugia is a major concern because survival of bed bugs will permit continued infestation. Residential areas can be very challenging as there are different materials, cracks and crevices that must be subject to and achieve a lethal temperature. Bed bugs have the ability to withstand elevated temperatures for short periods and these lethal temperatures have been previously underestimated (from Section 1). The egg stage is particularly resilient requiring extended times of exposure if minimal sub-lethal conditions are attained. Just as important is the rate of heating as bed bugs can detect lethal conditions and move to cooler areas (from Section 2). However, bed bugs respond differently when the air is heated versus heating by conduction and a delay in response will reduce chances that bed bugs will escape from interior of the room to exterior areas (from Section 3). Evaluating different areas during actual heat treatments will determine if concerns are warranted and if additional measures are necessary.

For this final set of experiments, we measured the rate of heating in two types of habitat, including an apartment and a house. While we know that air temperatures will be attained that will be lethal to bed bugs, at concern is the delay or interference of heating different articles within these habitats. Items of particular concern include upholstered furniture and other items with thermal mass, areas under carpeting and areas inside walls.

This part of the project entailed monitoring temperatures in these areas to determine the rate of increase in temperature and how long temperatures were maintained above the lethal threshold.

Methods

Temperature monitoring was conducted during two heat treatments. The first heat treatment took place in a single bedroom apartment, and a second treatment was conducted in a larger multi-level house. During the heat treatments, temperature dataloggers were placed in various areas within the apartment, including: in the general space, in between sofa cushions, inside the foundation of a mattress and underneath piles of clothes. During the heat treatment of the house, dataloggers were placed inside sofas and overstuffed chairs, and around the exterior perimeter of the room. Specific areas of the exterior perimeter included under carpeting, the wall-floor junction and inside the wall voids beside electrical junction or phone boxes. Temperatures were scanned every 30 – 60 seconds for the duration of the heat treatment.

Results

Average target increase in temperatures was 3.6 ^{o}C /hr, however the actual increase within the structures ranged from 4.6 to 13.2 ^{o}C/ hr in the apartment and 2.8 to 6.5 ^{o}C/hr for the house (Tables 4.1, 4.2). Particular areas of challenge included areas farther from the heating source(s), and areas where there is excessive thermal mass to overcome. Key areas such as upholstered furniture, under the carpet and under baseboards heated at close to the same rate as the air temperature (Figures 4.1 and 4.2).

Table 4.1. Thermal remediation of contents within an apartment and items that may create refugia for bed bugs

Location	Rate of Temperature Increase (°C)	Time above 45 °C (Min)	Time above 48 °C (Min)
Sofa	13.1	265	260
Sofa	13.2	254	253
Baseboard	8.9	242	191
Ottoman	13.3	266	262
Under Carpet	9.0	209	96
At exterior Wall	10.9	251	126
Upholstered Chair	11.3	156	243
Under TV	15.0	276	275
Mattress	10.6	257	181
Mattress	10.0	250	219
Under box	7.8	154	109
Inside Suitcase	9.7	241	210
Phonebook	6.9	155	117
Pile of bagged clothes	4.6	--	--
Under dresser	8.3	254	229
Baseboard	6.5	129	76

Table 4.2. Thermal remediation of contents and areas within a house that may create refugia for bed bugs

Location	Location within room	Rate of Temperature Increase (°C / hr)	Time above 45 °C (Min)	Time above 48 °C (Min)
Garage Apartment	Carpet Edge	4.6	211	43
	Under baseboard	5.3	171	40
	Behind wall plate	5.7	327	212
	30 cm under carpet	4.4	207	0
Basement Bedroom	Carpet Edge	4.0	0	0
	Under baseboard	2.8	0	0
	Behind wall plate	4.1	13	0
	30 cm under carpet	4.5	68	0
Master Bedroom	Air Temp	6.5	293	228
	Carpet Edge	4.5	144	97
	Under baseboard	4.9	96	29
	Behind wall plate	3.9	18	0
	30 cm under carpet	4.5	117	86
Sitting Room	Fabric Chair	5.7	164	120
	Leather Chair	5.0	151	134
	Behind wall plate	4.3	161	86

Figure 4.1. Heat up profile for upholstered articles in an apartment living room. The reference temperature (blue line) represents measurement of the room temperature.

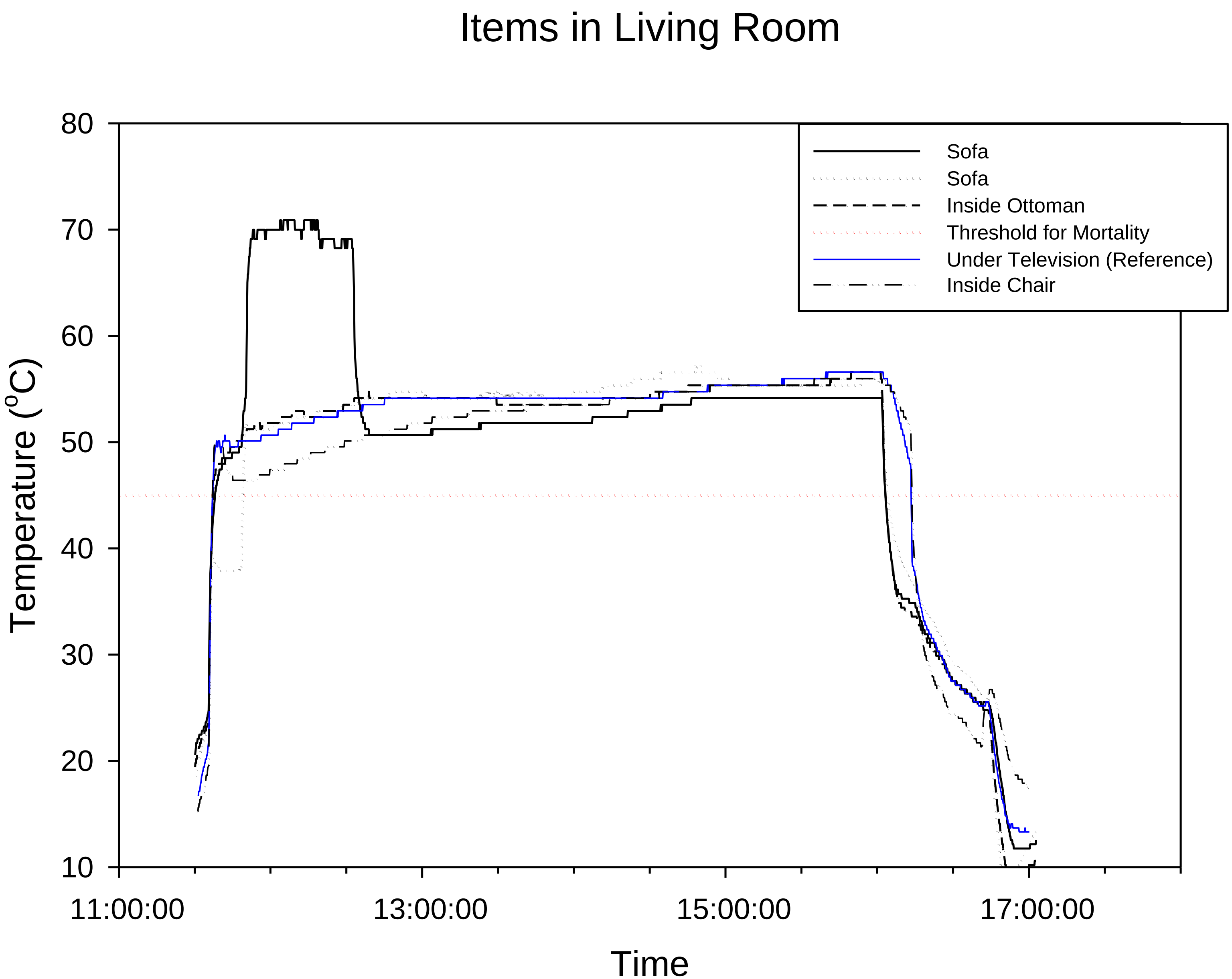
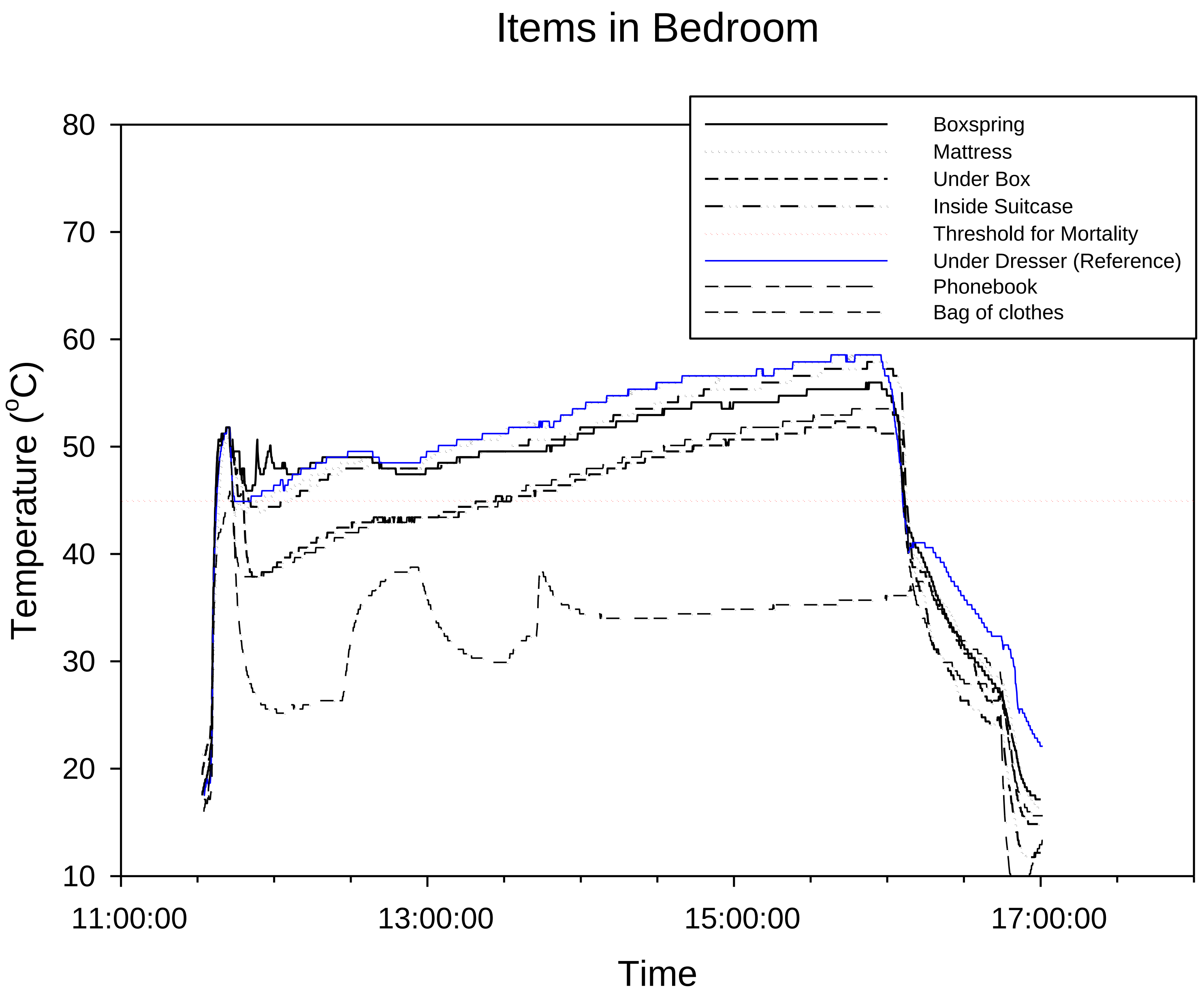


Figure 4.2. Heat up profile for different articles an apartment bedroom. The reference temperature (blue line) represents measurement of the room temperature.



In these figures, the blue line represents a reference to the room temperature. The greatest issue occurred from clothes that were bagged and piled on the floor (Figure 4.2), even with the bags moved twice during the heating, thermal mass precluded heating to threshold. With the exception of two sites, periods of exposure above the lethal threshold exceeded the minimum required conditions of 90 minutes at or above 48 ^{o}C (conditions to cause failure of emergence of eggs). As well, these two sites did not reach 50 ^{o}C , the minimum temperature for immediate mortality.

In the larger home, the slower rate of heating resulted in shorter periods above 45 and 48 ^{o}C and fewer times when 50 ^{o}C was achieved (Table 4.2, Figures 4.3 to 4.6). Possible reasons for this may be a result of outside temperatures and structural complexity of this particular house. In future treatments, it may be advisable to monitor the rate of temperature increase, not just the attainment of a target temperature.

Conclusions

1. Heat treatment to these two structures demonstrated that lethal conditions can be achieved, despite the requirement for hotter conditions to assure 100% mortality.
2. Variability in location and thermal mass within these two structures demonstrated challenges to heating all locales within the structure. Some of this variation can be overcome by identifying and manipulating areas resistant to heating (i.e., bags of clothes). Other areas may require additional monitoring to ensure lethal conditions were obtained.

3. In addition to monitoring for target conditions to be reached, monitoring rate of increase comparatively with air temperature will determine earlier in the process where additional heating should be focused.

Figure 4.3. Heat up profile for the living room above a garage in a house receiving a heat treatment to control bed bugs. The garage acted as a plenum for distributing air to the rest of the house.

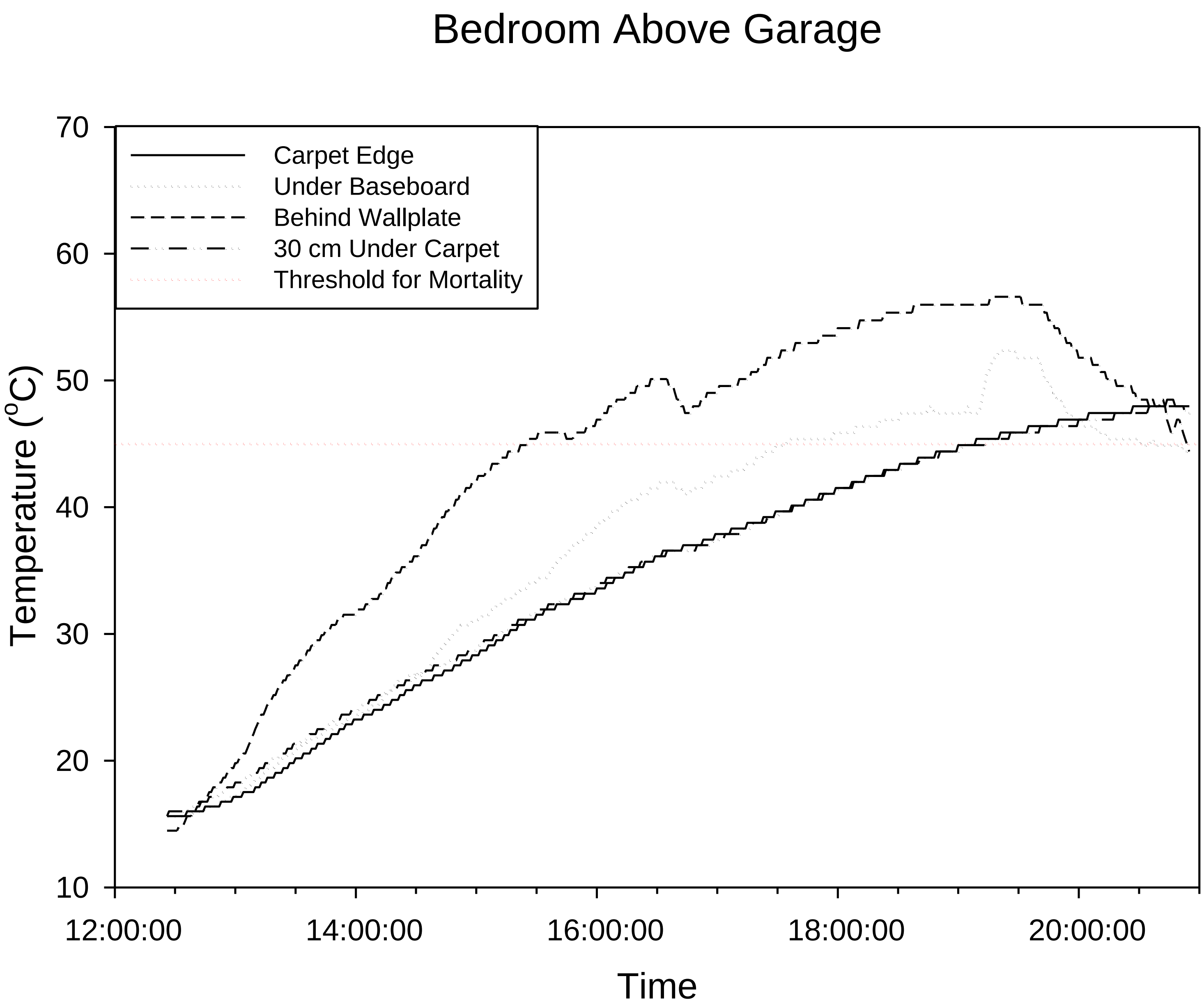


Figure 4.4. Heat up profile for the master bedroom in a house receiving a heat treatment to control bed bugs.

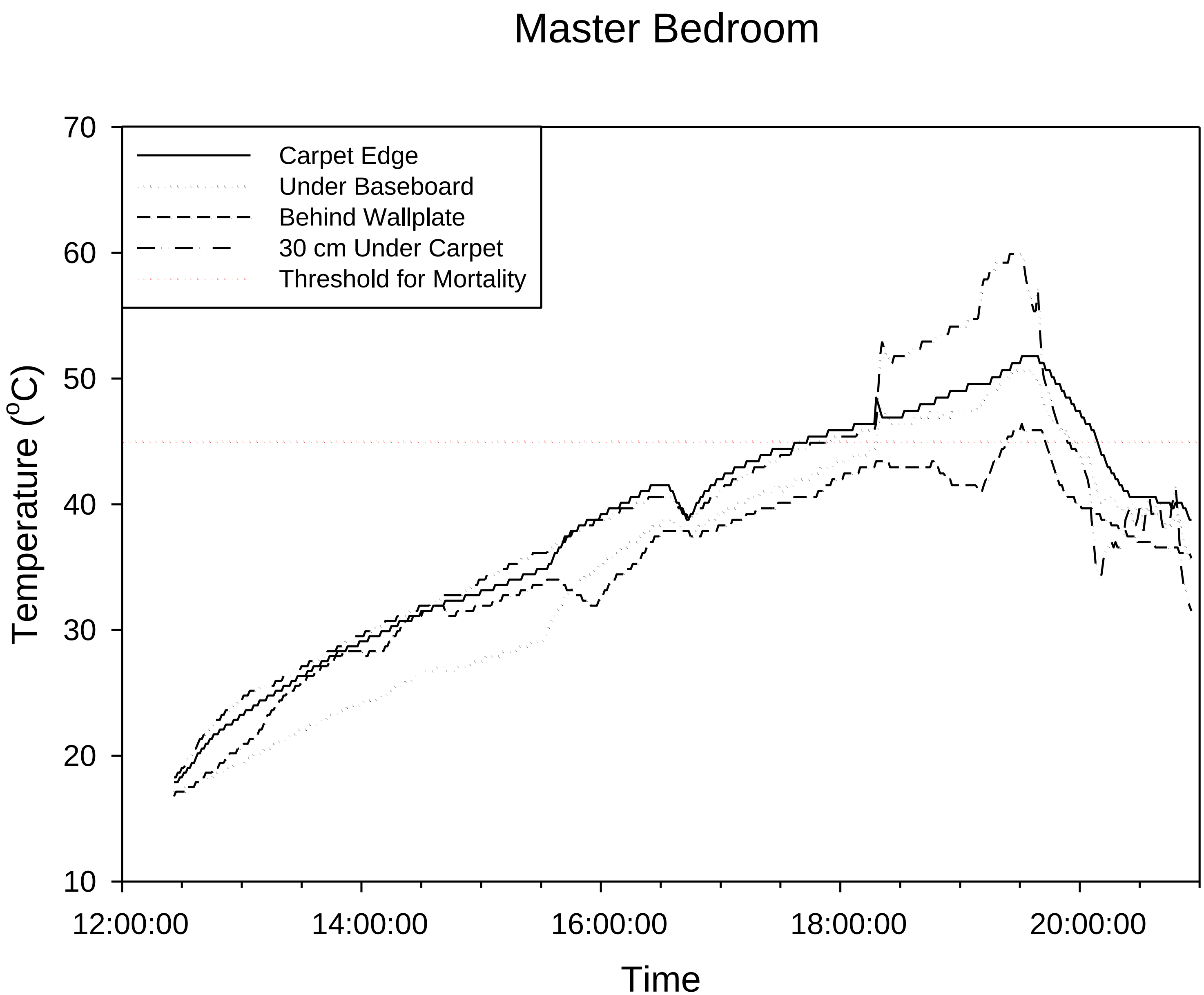


Figure 4.5. Heat up profile for the basement bedroom in a house receiving a heat treatment to control bed bugs.

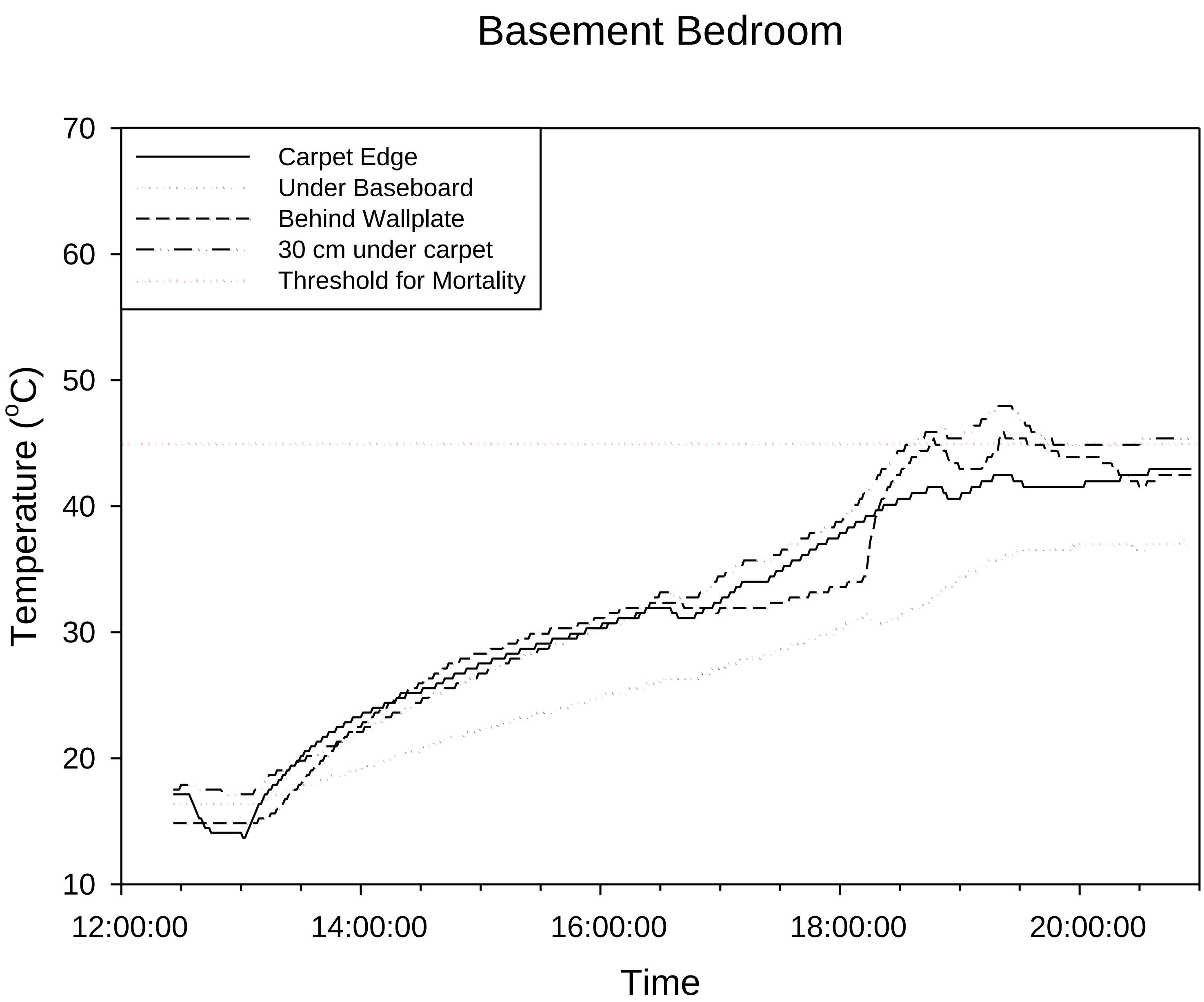
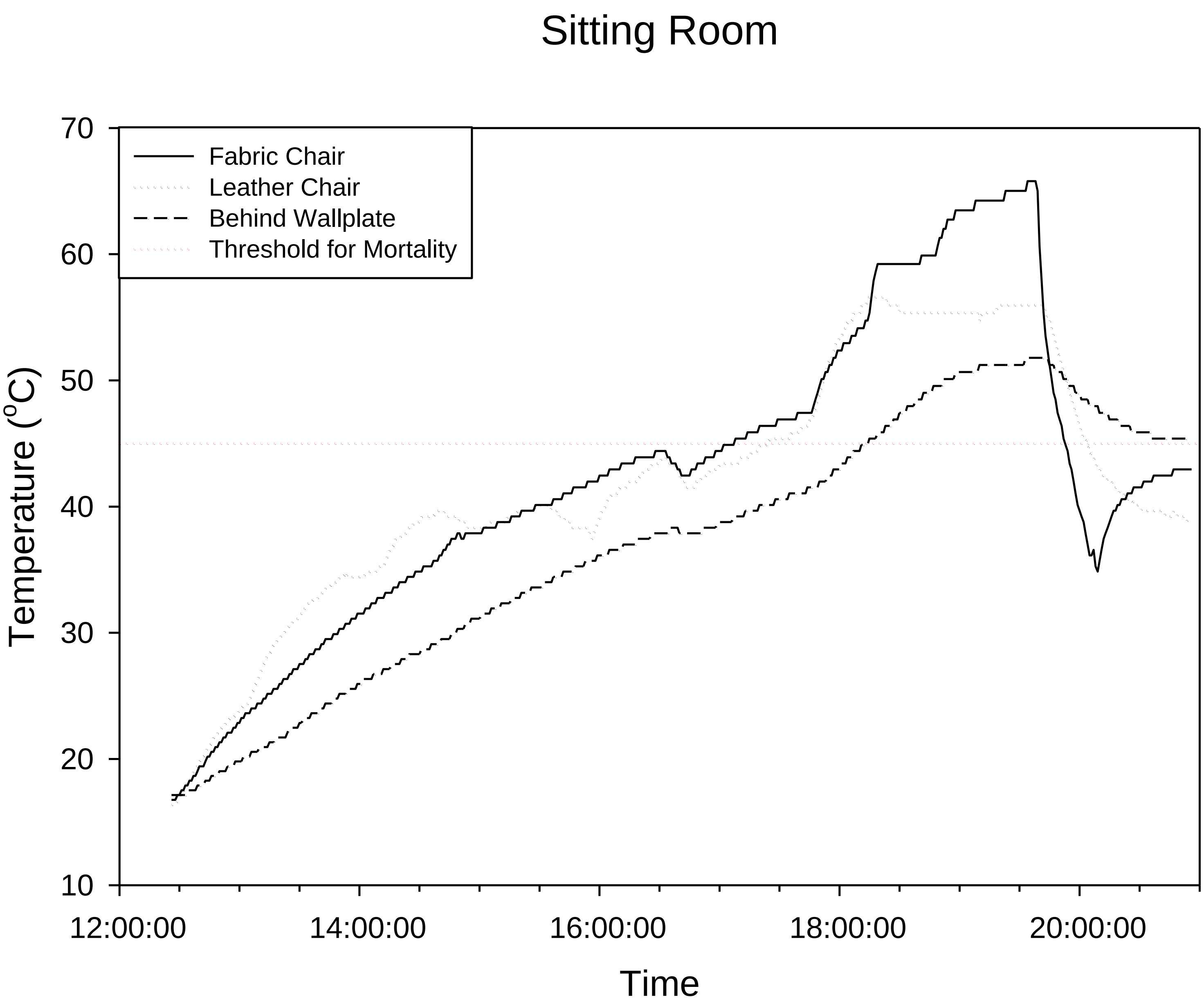


Figure 4.6. Heat up profile for the sitting room in a house receiving a heat treatment to control bed bugs.



The Use of Propane Fueled Heat Sources
as an Effective Means of Controlling Bed Bugs

This project demonstrated the ability for direct-fired propane heaters to effectively control bed bugs, in house and apartment situations. Although temperature tolerances of bed bugs are higher than originally thought, temperature monitoring in structures demonstrated that key areas in the living space can be heated to target temperatures lethal to bed bugs. Areas that may be perceived as challenging to achieve lethal conditions against bed bugs can be recognized, monitored and addressed during the heat treatment.

While bed bugs have a potential ability to escape a heating zone if exposed to conductive heating, most bed bugs will be exposed to convective heating first and their ability to escape a heating field is much reduced. For those areas where there is a concern for escape, the use of additional directed heating and supplemental insecticides will assist with any stragglers that may remain. This use of insecticide is greatly reduced compared to the ―chemical only" option.

There has been a shift in the methods used to deliver heat to living spaces and this forms a basis for future study. While direct-fired heaters still have application in specialty situations, there is a level of training and knowledge required by the service provider. Besides placement of heaters, there may be additional knowledge in connecting

Further study in a side-by-side experiment (slow heat up versus fast heat up) will be necessary to determine if there is a mechanism existing for resisting heat stress. Overall, the temperature requirement to control bed bugs is within reasonable parameters, though higher than originally expected.

1. Bed bugs do not immediately try to escape the heating field; many of them will actually forage or feed.
2. The difference in temperature between foraging / movement and escape is relatively close. This ensures many of the bed bugs will likely be contained.
3. Escape may occur and it is advisable to apply insecticides to perimeter areas where a gradient of temperature may occur.
4. In areas where a temperature gradient occurs, bed bugs will not simply run away. They will search in warmer areas, which may permit a lethal zone to develop around their harborage before they can escape.
5. Bed bugs were prone to wait in harborages when heat was delivered via air. Their threshold for escape was substantially higher.
6. Bed bugs showed a stronger propensity to stay with the harborage or would return to the harborage as air temperatures began heating theperipheral area.
7. While the distances were relatively small between the heated areas and refugia only 37.5% of bugs actually escaped. Measures to prevent bed bugs from escaping may include moving infested articles away from the walls and the use of directed air around the perimeter, before heating the interior.
    a. Heat treatment to these two structures demonstrated that lethal conditions can be achieved, despite the requirement for hotter conditions to assure 100% mortality.

b. Variability in location and thermal mass within these two structures demonstrated challenges to heating all locales within the structure. Some of this variation can be overcome by identifying and manipulating areas resistant to heating (i.e., bags of clothes). Other areas may require additional monitoring to ensure lethal conditions were obtained.
c. In addition to monitoring for target conditions to be reached, monitoring rate of increase comparatively with air temperature will determine earlier in the process where additional heating should be focused.

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Some of Dr. Stephen Kells resources on the web:

1. http://www.entomology.umn.edu/faculty/kells/kellscv.html
2. http://bedbugger.com/2008/04/03/more-bed-bug-research-stephen-kells-at-the-university-of-minnesota/
3. http://www.msnbc.msn.com/id/29984132/
4. http://www1.umn.edu/umnnews/Feature_Stories/Bloodthirsty_travelers3A_Bedbugs_are_biting_again.html
5. http://www.extension.umn.edu/distribution/housingandclothing/DK1022.html
6. http://www.ipmctoc.umn.edu/Travellers_prevent_hitchhiking_bedbugs.pdf

   (Google _kells bed bugs university minnesota‘ for more links and information)