How to kill bed bugs in portable items: unconventional non-chemical approaches

A Senior Research Thesis

Presented in Partial Fulfillment of the Requirements for graduation *with Research Distinction in Entomology* in the undergraduate colleges of The Ohio State University

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May 2016

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ABSTRACT

In the past decade, the bed bug, *Cimex lectularius* (Hemiptera: Cimicidae), has resurged globally as a household pest. Bed bugs currently are the most difficult and often the most expensive pest to eradicate, especially if an infestation is not detected early on. This has led many to resort to do-it-yourself (DIY) methods of bed bug control. The purpose of this study was to determine if a conventional microwave oven could be used to decontaminate sensitive items such as books that were infested with bed bugs. Three experiments were conducted using the EPM bed bug strain and a conventional microwave oven operating at full power. In the first experiment, groups of 10± bed bug eggs attached to filter paper were microwaved in open petri dishes. Results indicated that bed bug eggs did not hatch after being microwaved for 25 seconds. About 55% of the eggs hatched after 20 seconds and about 85–90% of the eggs hatched at 15 seconds or less. In the second experiment, bed bug nymphs and adults were microwaved individually in open petri dishes and mortality was assessed 24 hours later. The lethal times to kill 50% or 99% (LT50 or LT99, respectively) of the experimental population were calculated for the nymphal and adult stages. The LT99 values ranged from 26 seconds for adults to 44 seconds for first stage nymphs. The LT50 values ranged from 12 seconds for adults to 18 seconds for first stage nymphs. Recently fed bed bugs did not burst after being microwaved. In the third experiment, groups of 10 mixed stage nymphs and adults were placed in a small envelope that was inserted into an excavation made in the center of a paperback book which then was microwaved. These bed bugs died after 1 to 1.5 minutes of microwaving. Damage to the paperback book was observed after microwaving for 1.5 minutes.

ACKNOWLEDGEMENTS

Dr. Susan C. Jones for her help and guidance throughout the process of this project, and with her excellent revisions and edits to this thesis and presentations of this research project.

Joshua L. Bryant for his support and assistance with the completion of this research project.

Dr. David Shetlar for his help with revisions to the presentations of this research project.

Kara Baker for assistance with rearing and handling the bed bugs used in this study.

Nina Bogart for assisting in handling the bed bugs used in this research project.

Olimpia Ferguson for helping with various tasks to complete this research.

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CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 Basic Bed Bug Biology

The bed bug, *Cimex lectularius*, is an insect that feeds on the blood of humans, typically while they sleep. Bed bugs also can parasitize other warm-blooded animals, but they prefer humans as their primary hosts. Bed bugs have been a pest associated with human dwellings for thousands of years, but they were nearly eradicated from developed countries in the mid-20th century due to the introduction of DDT and other long-lasting pesticides (Romero et al. 2007).

Bed bugs have piercing-sucking mouthparts that they use to extract a blood meal from their host. It takes about 10 to 20 minutes for a bed bug to become fully engorged when extracting a blood meal (Centers for Disease Control 2013). Bed bugs typically feed about once a week but can feed less often. Bed bug adults can go as long as a year without a blood meal (Centers for Disease Control 2013). The nymphs require blood meals more frequently, and each nymphal stage requires a blood meal to molt (Centers for Disease Control 2013). Bed bug females also require a blood meal in order to produce eggs. (Rinehart and Siva-Jothy 2007).

When unfed, bed bugs have flat oval bodies that range in size from 1 mm as first stage nymphs to 7 mm as adults. It usually takes about 6 weeks for a bed bug to mature from egg through five nymphal instars to the adult stage (Rinehart and Siva-Jothy 2007).

A bed bug female typically can lay 4+ eggs a day and is capable of laying 200+ eggs over her entire life (Rinehart and Siva-Jothy 2007). Males mate with females via traumatic insemination, which involves piercing the female’s abdominal wall with sword-like genitalia. Sperm is then injected into the female’s blood, known as hemolymph, and then eventually make it to the ovaries (Stutt and Siva-Jothy 2001).

Bed bugs aggregate in cracks and crevices along box springs, mattresses, floors, and other locations where they can find safe harborages (Potter et al. 2006). Bed bugs release pheromones that cause them to aggregate (Gries et al. 2015). A sweet, musty smell can be observed in locations where there are heavy bed bug infestations. Since bed bugs can go through their life cycle in a short period of time in the presence of a host, a bed bug infestation can rapidly become very large (Potter et al. 2006).

1.2 Bed Bug Control Methods

Bed bug control methods range from professional treatment to do-it-yourself (DIY) methods. Professional pest management services are almost always needed to control bed bugs. Professional bed bug control methods include pesticide applications, heat treatment, and carbon dioxide fumigation. Pesticides that are commonly used to treat bed bugs such as pyrethroids often are not very effective against bed bugs. According to multiple studies, bed bugs have developed resistance to pyrethroid insecticides (Romero et al 2007, Fletcher and Axtell 1993, Zhu et al 2013). Pesticide resistance has necessitated alternative and multiple methods of bed bug treatment (Pereira et al. 2009).

There are various DIY products that can be used by an individual such as over-the-counter sprays, total release foggers, and sticky traps. The efficacy of many over-the-counter products is not well known and in some cases, many of these products are ineffective. A study was done on the efficacy of total release foggers, also known as bug bombs, which showed that bug bombs do not work against bed bugs (Jones and Bryant 2012). This study also suggested that bug bombs can make a bed bug infestation worse by causing bed bugs to scatter.

CHAPTER 2: UNCONVENTIONAL NONCHEMICAL APPROACHES

2.1 Introduction

During the past decade, the bed bug, *C. lectularius*, has become an increasingly common pest worldwide. The reasons for the bed bug resurgence include the banning of some pesticides, increased international travel, resistance to many pesticides and lack of public awareness about this pest. Bed bugs can multiply at a rapid rate, which makes them difficult to control. There is a social stigma as well as the psychological stress that goes along with having a bed bug infestation (Goddard and De Shazo 2012).

Bed bug control can be very costly and should involve multiple integrated pest management (IPM) tactics. Bed bug control can be very difficult, especially if an infestation is not stopped early on. Unfortunately, many people cannot afford professional treatment and rely on DIY methods, which can be ineffective and possibly hazardous to human health. DIY methods include laundering infested bedding and the use of over-the-counter products such as aerosol-based pesticides, sticky traps and total release foggers. Many of the over-the-counter products that are marketed for bed bug control are not very effective against bed bugs.

Microwave technology has been used in the medical and scientific community for a variety of purposes (Stuchly and Stuchly 1983). Microwave technology has been used to kill grain weevils and other pests infesting grains, seeds and various food items (Nelson 1973, Nelson 1996). In one study, a microwave oven was used to kill a drywood termite species that were put inside of wood blocks (Lewis et al. 2000.). In another study, a microwave was used to determine how microwave radiation affects a species of dermestid beetle (Dever et al 1990).

Microwave ovens operate by using radio waves at a certain frequency to agitate water molecules, causing them to generate heat (Vollmer 2004). This type of heat generation could be used to kill bed bugs. Heat treatments are often used for bed bug control and are most commonly used in whole house treatments (Pereira et al. 2009). A few products such as the "Packtite" bed bug oven and the "ZappBug Oven 2 Bed Bug Heater" have been marketed for heat-treating items infested with bed bugs. These products may not be affordable for low income individuals and their efficacy is not well known.

There is little to no information on the efficacy of microwaves against bed bugs. Hence, I conducted a preliminary experiment with groups of 20 bed bug adults that were placed inside of closed petri dishes and it took 10 seconds to kill these bed bugs. My research project then focused on determining how long it took a microwave oven to kill bed bugs under various conditions and whether a microwave oven could effectively decontaminate paper products such as books that were infested with bed bugs.

2.2 Research Objectives

The primary goal of this research was to determine the amount of time that it took a microwave oven to kill bed bugs. A secondary goal was to determine if a microwave oven could kill bed bugs hidden inside of paper products such as books. I also tested how long it would take for a book to incur damage after being microwaved.

2.3 Materials and Methods

This research was conducted under the supervision of Dr. Susan C. Jones at The Ohio State University Rothenbuhler Bee Lab located at 2501 Carmack Road, Columbus, OH 43210. A single Rival Brand microwave oven (700 watt, 2450 MHz) was used for all research trials. This is a standard size microwave oven that is commonly used by the general public in kitchens. The power scale is from 1–10 and the maximum power level was used for all trials.

Controls were established for all experiments, with the bugs placed in the microwave for a short amount of time, but without the microwave being turned on. The purpose of this was to take into account any bed bug mortality caused by the handling process.

The EPM bed bug strain was used in all experiments. This strain was collected in 2010.

2.3.1 Experiment 1: Microwave Effects on Egg Hatch

The first experiment involved bed bug eggs. The eggs were obtained by placing ≥75 female bed bug adults and 5 to 8 male bed bug adults together in a closed and vented petri dish. The females laid their eggs on a filter paper substrate that was placed inside of each petri dish. After a significant amount of egg laying occurred, the adult bed bugs were removed, and the filter paper was carefully cut into small pieces such that each piece had approximately 10 attached eggs, and these 10 eggs comprised a replicate. Three replicates were used for each microwaving time which ranged from 0 seconds to 35 seconds, at 5 second intervals. Each filter paper piece with eggs was placed in a small open petri dish while it was microwaved. Afterwards, each egg replicate was placed into an individual well of a 24-well plate (surface area 2 cm^2) for observations of hatching over the next 2 weeks.

An ANOVA, which is a statistical test for analysis of variance and is used to show differences between two or more means, was also performed on the egg data. A Tukey means separation test was used to determine means that significantly differed from one another.

2.3.1 Experiment 2: Microwave Effects on Exposed Nymphs and Adults

The second experiment involved bed bug nymphs of all stages and adults regardless of feeding status. Twenty replicates were tested with each replicate consisting of 1 bed bug. Each bed bug stage was subjected to a microwaving time ranging from 0 seconds to 30 seconds, with time intervals of 2 to 3 seconds. After microwaving, each bed bug was transferred to an individual well of a 24-well plate and it was monitored for at least 24 hours.

A test was also performed to determine if recently fed adult bed bugs could burst or pop when microwaved since blood can stain paper products. Groups of 20 mixed gender bed bugs were tested. They were placed into a 250 ml beaker and then microwaved for 5 minutes.

Trials also included recently molted virgin males and virgin females to account for any potential popping that could be caused by traumatic insemination. To obtain virgin adult bed bugs, fifth stage nymphs were separated and fed individually until they molted into adults. Once molted, the virgin bed bug adults were separated into groups of males only and females only. Groups of 20 males and 20 females, either virgin or non-virgin, were tested. Each group was first given a blood meal then promptly placed into a 250 ml beaker and microwaved for 5 minutes. Observations were made immediately after the bugs were microwaved to determine if there was any popping. The lethal time in seconds to kill 50% (LT50) and to kill 99% (LT99) of the experimental groups with 95% confidence intervals were calculated for all stages of nymphs and adult bed bugs.

2.3.3 Experiment 3: Microwave Effects on Nymphs and Adults Inside of Books

A paperback book, the Proceedings of the 2010 National Conference on Urban Entomology (NCUE), was used for all trials. In order to avoid bed bugs dying from the books becoming heated after multiple microwaving trials, three NCUE books were alternated and each was reused only when completely cooled.

A small rectangular excavation (4.3 by 3.7 by 0.7 cm, L x W x H) was made in the center of all three of the NCUE books in the same location. A small envelope, containing 10 mixed staged nymph or adult bed bugs then was inserted into the excavation for microwaving trials. Microwaving times were 0, 30, 60, and 90 seconds; three replicates per time were performed for the nymphs and adults.

A test was performed with an additional NCUE book to determine the amount of time it would take for the book to incur damage from microwaving. The book was microwaved for up to 10 minutes total. The microwave was stopped every 2 minutes and the book was inspected during these 2 minute pauses to assess damage.

2.4 Results

In the first experiment, it was found that 25 seconds was the required amount of time to prevent bed bug eggs from hatching (Figure 1). At 20 seconds of microwaving, approximately 55% of the eggs did not hatch (Figure 1). At 15 seconds and less, the hatch rate was 85–90% (Figure 1).

In the second experiment, the earlier stage bed bugs took the longest amount of time (44 seconds) to achieve maximum mortality, whereas the adults took the shortest amount of time (26 seconds) (Figure 2). The LT99 value for the first stage nymphs (44 seconds) was especially high compared to the other stages of bed bugs (18–26 seconds) (Figure 2). In general, an inverse trend for bed bug size and microwave time was observed for both the LT50 and LT99 values (Figure 2). The largest bed bugs (adults, fifth stage and fourth stage nymphs) required the shortest amount of microwaving time before dying) and the first stage nymphs took the longest to die in the microwave (Figure 2).

In trials to determine if recently fed bed bugs could burst when microwaved, only 2 of 20 bed bugs burst. Blood stains were observed on the side of the beaker. Traumatic insemination was not a cause as no bugs burst in trials with virgin males or virgin females.

For the third experiment, it took between 1 minute to 1.5 minutes to kill all nymphs and adults hidden inside of the paperback book. The NCUE books were slightly damaged after being microwaved for 1.5 minutes. A "torture test" was done with an additional NCUE book to determine the type of damage that a book could incur while being microwaved. Significant damage such as melted binding, crinkly pages and burn marks were observed and the book was radiating heat after being microwaved for 2 minutes.

2.5 Discussion

Based on the results of this study, it was found that a microwave was capable of killing exposed bed bugs of all stages in a short amount of time (20-44 seconds). Bed bugs eggs failed to hatch after 25 seconds of microwaving (Figure 1). At 20 seconds of microwaving time, 55% of the eggs hatched, whereas at ≤15 seconds, about 85–90% of the eggs hatched. The composition of a bed bug egg could be a factor in terms of how well microwave radiation can penetrate the layers of the egg.

Longer microwaving times were required to kill earlier stage nymphs compared to adults, possibly be due to their small size. Older stage nymphs and adults have more body mass and fluids for the microwave radiation to penetrate. The longer microwaving times for the earlier stage nymphs are reflected in the LT50 and LT99 values (Figure 2). Some variation in

LT50 and LT99 values was observed despite the trend wherein earlier stage nymphs required longer microwaving times to die (Figure 2). The LT50 and LT99 values for adults were lower than the values for 4th and 5th stage nymphs (Figure 2). Despite these differences, there appears to be an inverse relationship with size (Figure 2).

The test to determine if recently fed bed bugs could burst was performed because the blood from a recently fed burst bed bug could stain the pages of a book. During one of the trials which involved mixed gender adult bed bugs, 2 of the 20 bed bugs burst and there was a noticeable streak of blood on the beaker that contained this replicate after it was microwaved.

In the third experiment, it took 1.5 minutes to kill all bed bug nymphs and adults hidden inside of an NCUE book. This time duration is significantly higher than the LT50 and LT99 values (Figure 2) of experiment 2 and likely are caused by the book absorbing some of the microwave radiation. Preliminary trials were performed with a hardback book and it took about 1 minute to kill all bed bug nymphs and adults hidden inside of it. The thickness and consistency of a book are factors when considering microwaving times to kill any hidden bed bug nymphs and adults.

It took 1.5 minutes of microwaving to cause some damage to the three NCUE books used in the bed bug trails and 2 minutes was enough time to significantly damage an NCUE book. A book must be able to withstand the microwaving duration to kill any bed bugs hidden inside of them yet not be damaged. Therefore, books should be microwaved for the least amount of time that causes bed bug mortality.

A microwave oven does have some potential as a DIY method for killing bed bugs hidden inside of books but it also could be a potential hazard. In a scenario involving a microwaving treatment of a bed bug-infested book, the book should be inspected to ensure that it has no metallic parts. Spiral binding, gold-leaf pages, and any other metallic parts on a book can be a fire hazard because metal objects are likely to spark when they are microwaved (Vollmer et al. 2004).

2.6 Conclusions and Directions for Future Study

In conclusion, this study has demonstrated that a conventional microwave oven has some potential as a tool for DIY bed bug control since microwaves quickly killed bed bugs of all stages of bed bugs in open petri dishes (20–44 seconds) and bed bug nymphs and adults hidden inside of a paperback book (1.5 minutes). Damage to paperback books that are microwaved is highly likely, therefore, books should be microwaved for the minimal amount of time to achieve maximum bed bug mortality. Microwave technology has been used in other pest control applications and experiments, and could potentially be useful for future bed bug control applications based on the results of this study.

Future research should be done to provide microwaving time recommendations for hardback books and books of various sizes and shapes as well as for bed bug eggs inside of books. Further studies can also be done on different microwave power settings, different types of microwave ovens and with other appliances such as kitchen ovens that could potentially be used to kill bed bugs.

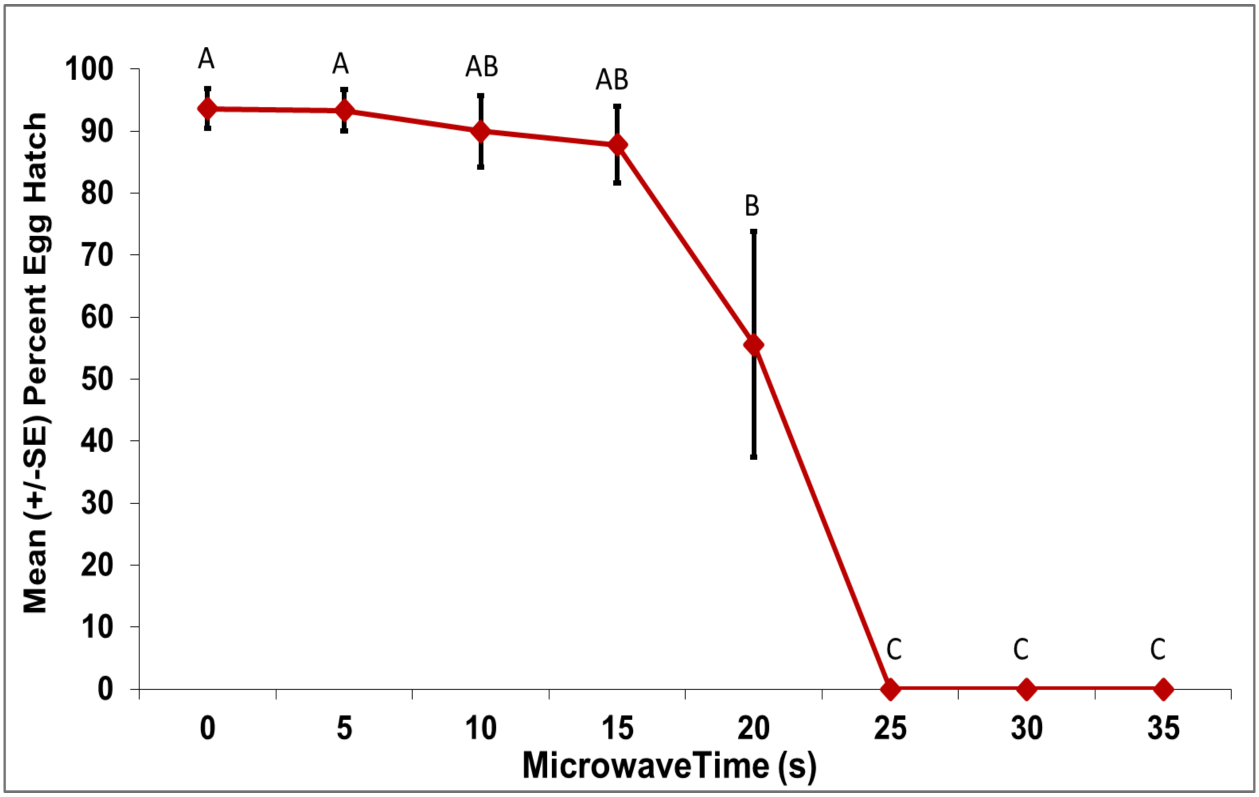

Figure 1. Percent (mean ± SE) of bed bug eggs that hatched after being microwaved for various times. Means with different letters are significantly different based on post-hoc pairwise Tukey comparisons.

Stage	Time (s)	
	LT_{50} (95% CI)	LT_{99} (95% CI)
1st stage nymph	**18.3** (16.4 – 20.3)	**43.7** (38.3 – 52.4)
2nd stage nymph	**13.0** (11.9 – 14.3)	**25.3** (22.4 – 30.1)
3rd stage nymph	**13.1** (11.8 – 14.4)	**25.1** (22.2 – 30.2)
4th stage nymph	**11.1** (10.1 – 12.0)	**18.0** (16.3 – 20.1)
5th stage nymph	**9.8** (8.8 – 10.7)	**21.5** (19.4 – 24.6)
Adult	**12.0** (11.1 – 13.0)	**25.6** (23.0 – 29.5)

Figure 2. Lethal time to kill 50% and 99% of each bed bug stage using a microwave oven.

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