BEHAVIORAL ECOLOGY AND CONTROL OF BED BUGS, *CIMEX LECTULARIUS* L., IN MULTI-FAMILY HOUSING COMMUNITIES

By

Richard Alan Cooper

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ABSTRACT OF THE DISSERTATION

Behavioral ecology and control of bed bugs, *Cimex lectularius* L.,

in multifamily housing communities

by Richard Alan Cooper

Dissertation Director:

Changlu Wang

After nearly a fifty-year absence, the bed bug *(Cimex lectularius* L.) has reemerged as a very important urban pest affecting persons of all economic strata. My research was conducted in affordable housing communities for the elderly. These communities suffer disproportionately high infestation rates compared to other housing communities. In my first study, I evaluated the accuracy of commercially available canine scent detection teams to detect bed bugs in apartments and compared the results to detection using pitfall-style traps. The mean detection and false positive (false indication of bed bugs) rate among 11 teams was 44% (10 – 100%) and 15% (0-57%), respectively. In comparison to dogs, placement of traps detected 93% of the infested apartments. The poor performance of canine teams under field conditions reveals the need for further investigation of factors affecting the accuracy of canine detection. In my second study, I used mark-release-recapture (m-r-r) technique to study bed bug movement within and between apartments. I demonstrated that bed bugs travel extensively throughout apartments regardless of their release location (at or away from host feeding sites). Marked bed bugs were also recovered in one or more apartments neighboring 4 of 6 m-r-r

units 14-15 days after release. My third study evaluated the effects of various interventions, including mass trapping with traps, in apartments with low-level (≤ 10 bed bugs) infestations. In the first experiment, bed bugs were eliminated without any control intervention (other than traps) in 96% of the apartments with newly identified bed bugs and 96% of those that had recently been treated after 22 weeks. A second experiment demonstrated that the mass trapping contributes to the control of low-level populations. Last, I developed and implemented a model integrated pest management (IPM) program that successfully managed bed bugs at the community-level in an affordable housing community where previous control efforts had failed. The infestation rate was reduced from 15 to 2% within 12 months. Of the existing bed bug infestations, 72% were detected during proactive community-wide inspection of apartments. Proactive inspections and implementation of a rigorous elimination protocol played a major role in the success of the program.

DEDICATION

In memory of my father Theodore (Ted) H. Cooper. I wish you were here with all of us to share this accomplishment. I know you are with me in spirit.

ACKNOWLEDGMENTS

Completing my PhD has been a personal dream of mine since I received my Master's degree in Entomology 25 years ago. Achieving this goal would not have been possible without the support and assistance of many people whom I wish to recognize and thank.

I would like to thank my work family at Cooper Pest Solutions and BedBug Central for the sacrifices they made on my behalf. Attaining my PhD would not have been possible without their support. My brother Phil, made it possible for me to pursue my PhD, just as he did when I left to complete my MS degree. My return to Rutgers also would not have been possible without the support from the leadership and management teams, along with the rest of the staff at Cooper Pest Solutions and BedBug Central. Reaching this goal took longer than I expected and placed a great deal of pressure on my Cooper Pest and BedBug Central families. I owe you all a great debt of gratitude and look forward to my return. Thank you!

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I enjoyed being part of the graduate student community and sharing the Graduate experience with my fellow graduate students. I especially enjoyed my friendship with Lauren Widener, who I watched grow from a tentative young graduate student into an amazingly confident and accomplished scientist. I know you will do amazing things!

Finally, and most importantly, I would like to thank my family, starting with my wife and best friend, Louise, along with our two wonderful daughters, Julia and Andrea who encouraged me to go back and pursue my personal dream. I thank Louise and my girls for dealing with the many hours I spent in front of a computer, and for often being preoccupied by my research. Louise thank you for always having my back, and knowing me better than I know myself. I could not have done this without your love, patience, and support. I know it felt like I would never finish. My brother, Phil has always made personal sacrifices in order for me to continue my education. I can never thank you enough. Finally my mom, Sybil, is one of my biggest fans and has always been there for me, her constant love and support are greatly appreciated. Although my father, Ted Cooper, is no longer with us I know he would be proud and I dedicate this dissertation in his memory.

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INTRODUCTION

Much of what we know today regarding the behavioral ecology of bed bugs comes from early researches including Mellanby (1932, 1939), Rivnay (1932), Kemper (1930, 1936), Johnson (1941), Omori (1941), and Marx (1955) whose works have been summarized in Usinger's Monograph of the Cimicidae (Usinger 1965). Since then research on bed bugs has been scant. Levinson and Barr Ilan (1971) added to our understanding of chemical ecology in bed bugs in the 1970s and Newberry and Jansen (1986) contributed some information regarding the population distribution of bed bugs in heavily infested huts in Africa. Over the past fifteen years, the resurgence of bed bugs has sparked a renewed interest in the study of bed bugs from both a basic and applied perspective. A number of excellent review articles have been published on the resurgence and control of bed bugs (Doggett and Russel 2005, Reinhardt and Siva-Jothy 2007, Doggett et al. 2012, Koganemaru and Miller 2013a), clinical importance (Delaunay et al. 2011, Goddard and deShazo 2012, Doggett et. al. 2012), stress tolerance (Benoit 2011), chemical ecology (Weeks et al. 2010), and sexual conflict in bed bugs (Reinhardt et al. 2014). Still, many gaps exist in our understanding of bed bugs.

My dissertation focuses on the ecology and control of naturally occurring bed bug infestations in apartments, a topic where little information is currently available. I was very interested in making strides in our ability to detect bed bugs present only in small numbers and to expand our understanding of the behavioral ecology of bed bugs as it relates to their detection, management, and more importantly, the eradication of infestations in affordable housing communities.

All of my field research was conducted in affordable housing communities for elderly and disabled individuals. I chose to conduct my field research in this setting for a variety reasons: 1) these communities are at risk for high infestation rates; 2) current pest management efforts are typically inadequate; 3) the housing style is simple compared to family-style housing and provides a very consistent research model, most apartments are studio or one bedroom units located in high-rise buildings, and 4) children and pets are rarely present allowing for less disruption of experiments.

In Chapter Two, I evaluate the accuracy of canine scent detection for the detection of bed bugs in apartments. I was particularly interested in determining the level of accuracy of inspections conducted by commercial canine scent detection companies. I hypothesized that; 1) the average detection rates would be significantly lower than 95%, and 2) the average false positive rates would exceed 10%. A secondary objective was to determine which of three methods; 1) canine scent detection, 2) visual inspection, or 3) placement of pitfall-style interceptor traps, is most effective for detecting bed bug activity.

In Chapter Three, I use a mark-release-recapture technique to investigate bed bug movement within and between apartments. Experiments were conducted in both occupied and vacant apartments to determine if active dispersal of bed bugs from mark-release units to neighboring units occurs, as well as to study the movement of bed bugs released at and away from host sleeping areas. The mark-release-recapture method was also used to estimate population size. Finally, longevity of bed bugs in the absence of a host was investigated in a vacant apartment.

In Chapter Four, I examine the effect of various interventions, including mass-trapping with pitfall-style interceptor traps, on low-level bed bug populations in apartments. One experiment investigates changes in bed bug counts over 40 weeks in occupied apartments that had; 1) never been treated, 2) recently been treated with no further treatment, and 3) recently been treated with continued treatment. The second experiment investigates the impact of mass trapping with pitfall-style interceptor traps on bed bug counts over 16 weeks. The hypothesis is that bed bug counts will be significantly reduced in apartments with low-level infestations through continued mass trapping alone compared to control apartments where traps were not continuously present.

In Chapter Five, I develop, implement, and evaluate a model community-wide bed bug IPM program for an affordable housing community. The IPM program included an educational component for residents and housing staff, a baseline inspection to identify unreported infestations, treatment of infestations, implementation of a unique "elimination" protocol, and community wide inspections at 6 and 12 months to evaluate the effectiveness of the program and to identify new unreported bed bug infestations. The objectives of the study are to: 1) reduce the apartment-complex-wide infestation rates by at least 70% within 12 months, and 2) reduce the amount of pesticides applied for the control of bed bugs over the course of the study.

CHAPTER ONE

Literature Review

History and Background of the Bed Bug Resurgence

The common bed bug, *Cimex lectularius* (L.), is a hematophagous ectoparasite belonging to the order Hemiptera (true bugs), suborder Heteroptera, family Cimicidae. The family Cimicidae is divided into 24 genera consisting of 110 species (Henry 2009). Most cimicids feed primarily upon the blood of birds and bats (Usinger 1966), however there are three species known to commonly feed upon humans. One of the three, *Leptocimex boueti* (Brupt), feeds primarily on bats but also regularly feeds upon humans and occurs only in West Africa. The other two, *Cimex hemipterus* F. (the tropical bed bug) and *Cimex lectularius* (the common bed bug) use humans as their primary host. The tropical bed bug is most commonly found along the equator, as its name suggests, while the common bed bug occurs worldwide and is ubiquitous in nature (Usinger 1966).

The association of bed bugs with man is an ancient one dating back at least 3,500 years, based upon fossilized remains recovered in Armana, Egypt (Panagiotakopulu and Buckland 1999). References to bed bugs can also be found in early writings from the ancient Egyptians, Greeks, and Romans as well as in religious writings in the Talmud and New Testament (Busvine 1966, Pinto et al. 2007, Potter 2011). There are some who believe bed bugs first used birds as a host before switching to humans (Weidner 1958). However, the most commonly accepted hypothesis is that bed bugs originated in caves, where they fed on bats before switching to humans as their primary host (Usinger 1966). Recent molecular studies by Balvin et al. (2012) and Booth et al. (2015) support the hypothesis that the bed bugs which plague mankind today originally fed upon bats and followed humans as they moved out of caves. As the humans expanded their geographic range from the Mediterranean region (Usinger 1966), into Asia, Europe, and eventually the Americas, bed bugs followed using human trade routes to follow their host around the world (Kemper, 1936, Usinger 1966, Potter et al. 2011).

By the early 1800's severe infestations were recorded in English colonies located in seaport towns of North America (Pinto et al. 2007). Technological advancements such as the heating of homes during cold weather enabled bed bugs to breed within human structures year round. This coupled with dense living quarters and low hygiene standards enabled bed bugs to flourish, especially among the poor during the 1800's (Potter et al. 2011). However, even as early as 1603, the non-discriminatory nature of bed bugs was recognized by the Italian naturalist Ulisse Aldrovandi, who noted that bed bugs were a pest infesting the homes of both the rich and the poor.

Some ancient references dating as far back as 400 BC espouse the use of bed bugs for medicinal purposes (Potter et al. 2011), however most recognize bed bugs as a scourge upon humanity (Busvine 1966, Panagiotakopulu and Buckland 1999, Potter et al. 2011) which gave rise to businesses specializing in the control of bed bugs in 17th century England (Cowan 1865). During the first half of the 20th century bed bugs were not only rampant in homes but were also commonly found in non-residential settings, including moving vans, schools, businesses, theaters, and various modes of transport (Potter et al. 2011). The need for pest control services to control bed bugs continued to grow until the middle of the 20th century when bed bugs were virtually eradicated from developed countries through the widespread use of modern synthetic insecticides such DDT and malathion, along with improvements in housekeeping and personal hygiene practices (Pinto et al. 2007).

After nearly a fifty-year hiatus, bed bugs have once again emerged as a global pest of great social, economic, and public health importance (Pinto et al. 2007, Doggett et al. 2012). The resurgence of bed bugs began around the turn of the century in Australia, the UK and United States (Boase 2000, Krueger 2000, Doggett et al. 2003, Cooper and Harlan 2004). Within five years bed bugs had been reported in all 50 states in the U.S. (Cooper and Harlan 2004). Among 509 pest control professionals surveyed in 2007, 91% reported that they had encountered bed bug infestations in the previous two years (Potter et al. 2008a). By 2010 bed bugs were once again being reported in lodging facilities, homes, schools, offices, health care facilities, movie theaters and various modes of public transportation (Potter et al. 2011), as was the case during the first half of the 20th century. Within a little over 10 years since the bed bug resurgence, conservative estimates by Doggett et al. (2102) place the economic impact of the resurgence in Australia at AU $200 million and $3 billion in the U.S. Bed bugs are now considered to be among the most difficult and important urban pest facing today's pest management professional (Pinto et al. 2007, Potter et al. 2013a).

The precise cause for the resurgence of bed bugs in the U.S. is still unknown and is probably the result of a combination of different factors (Boase 2008, Romero et al. 2007a, Potter 2005, Cooper 2011). Szalanski et al. (2008) suggested bed bug populations isolated on birds in poultry houses may have served as a domestic source for the resurgence based upon similarities in mitochondrial haplotypes shared between bed bugs in poultry houses and human structures. More recent research provides strong evidence suggesting the resurgence of bed bugs in the United States resulted largely from a number of independent introductions of bed bugs into the country from other parts of the world (Booth et al. 2012, Saenz et al. 2012). Regardless of the source, once introduced into human dwellings, factors such as; lack of public awareness, lack of early detection, cost-prohibitive pest control for the economically disadvantaged, inadequate pest management, and insecticide resistance have fostered the successful spread of bed bugs within living communities and throughout society (Pinto et al. 2007, Reinhardt and Siva-Jothy 2007, Cooper 2011, Wang and Cooper 2011).

Bed Bug Biology and Life History

The common bed bug is a dorsoventrally flattened, wingless, temporary ectoparaiste of humans. In addition to humans, bed bugs will readily feed on the blood of a wide variety of other warm blooded animals (Usinger 1966) but virtually nothing is known regarding host preference or host switching in naturally occurring infestations. Bed bugs undergo gradual metamorphosis and are obligate blood feeders, requiring blood for nymphal development and reproduction in adults, and relying on endosymbiotic *Wolbachia*, to provide vitamin B, known to be deficient in the blood (Hosokawa et al. 2010).

Developmental time, fecundity and longevity of bed bugs varies widely based upon temperature (Johnson 1941, Usinger 1966), food availability (Kemper 1930, Johnson 1941, Omori 1941, Polanco et al. 2011c), and food quality (Johnson 1937, Barbarin 2013). At 26 ºC approximately six days are required for eggs to hatch and another 35–40 days to progress through five nymphal instars and reach adulthood (Polanco et al. 2011a). Adults, live for approximately 4.5 months (Busvine 1980) but have been reported to live more than one year at low temperatures (10 °C) or during periods of starvation, in the absence of a host (Johnson 1941, Omori 1941).

The various developmental stages of the bed bug are illustrated in Fig. 1.1. Females deposit approximately 2-3 eggs per day, from which nymphs will emerge to begin the bed bug life cycle. The eggs which are oval in shape, ivory in color, and about 1 mm in length, are firmly cemented to a substrate by the female (Usinger 1966).

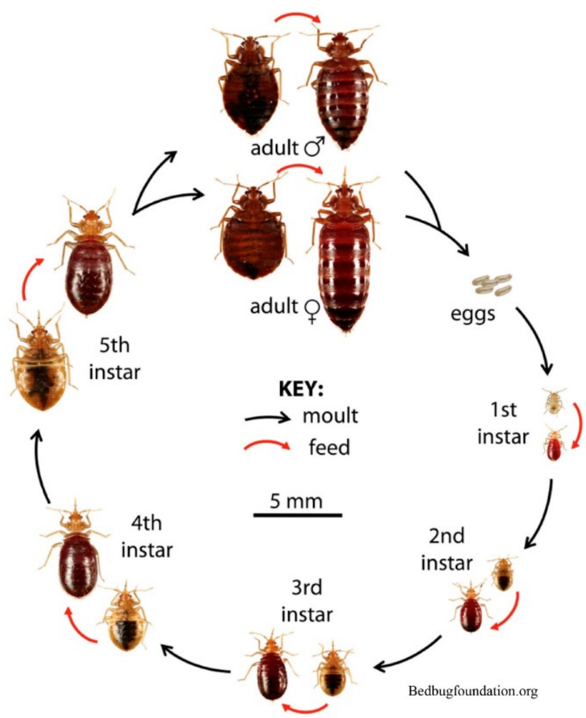
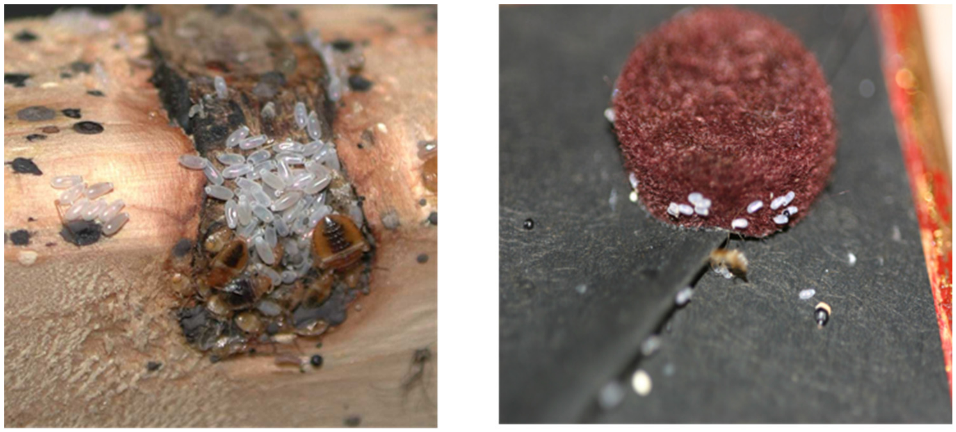


Figure 1.1. Bed bug life cycle

Although deposited singly, eggs are often found clustered within bed bug refugia close to host sleeping and resting areas (Fig 1.2A) but can also be found in seemingly unpredictable locations away from visible bed bug aggregations and host feeding sites (Fig 1.2B) (Pinto et al. 2007).



A. B.

Figure 1.2. Bed bug eggs. A. shows eggs clustered on the frame of a box spring, B. shows eggs on the back of a picture frame.

First instar nymphs are approximately 1 mm in length, light in color (light tan) and somewhat ` (Usinger 1966). Nymphs undergo five developmental instars, requiring at least one blood meal during each stage before progressing to the next (Usinger 1966). With each molt, nymphs become progressively larger and darker in color. Adult bed bugs are approximately 5 mm in length and reddish (mahogany) brown in color. The abdomen of the adult male narrows distally while the abdomen of the adult female is broadly rounded at the terminal end, providing a morphological feature which can be used to easily differentiate the two sexes Fig 1.3. Adult females can be further distinguished from adult males by the presence of an ectospermalege which can be visibly observed on the right side of the 5th abdominal sternite of the female, a feature lacking in adult males (Usinger 1966) (Fig 1.4).

Figure 1.3. Comparison of adult male and female bed bug; male (left) with terminal end of the abdomen tapered, female (right) with terminal end of abdomen broadly rounded (photo taken by Stephen Doggett).

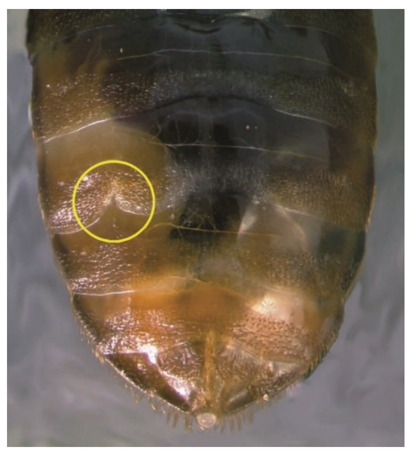


Figure 1.4. Adult female ectospermalege circled in yellow (photo by Margaret Lehnert)

Bed bugs typically live in aggregations located in narrow cracks and crevices, within a few meters of their host (Johnson 1941, Reinhardt and Siva-Jothy 2007). Bed bug harborages are characterized by the presence of bed bugs of all stages (eggs, nymphs and adults) along with feces, and exuvia. While they can be active at any time of day, bed bugs are usually inactive during the daytime hours, hiding within harborages that tend to be discretely located in areas that are out of the sight, where they are not easily detected. Foraging activity and food acquisition, usually occurs at night when their host(s) are less active or sleeping, which limits the likelihood that they will be detected while acquiring a blood meal (Usinger 1966, Romero et al. 2010a). Under laboratory conditions, Romero et al. (2010a) demonstrated that locomotor activity is endogenously controlled by circadian clocks and can be entrained by light conditions. They also demonstrated that bed bugs starved for one week were more active than those starved for five weeks, indicating that activity is also affected by feeding history. Romero et al. (2010a) speculated that in the prolonged absence of a host, bed bugs switch from regular exploratory forays to host-stimulus dependent foraging activity, to conserve energy during periods of time when food is unavailable.

Food acquisition is controlled by a variety of exogenous (light, temperature, time of day, chemical kairomones) as well as endogenous (life-stage, gender, nutritional state) factors (Suchy and Lewis 2011). Bed bugs can locate their host from a distance of up to 1.5 m using carbon dioxide (CO_2) as a long distance cue (Marx 1955), while temperature and chemical kairomones are attractive over a much shorter range (Rivnay 1932, Aboul-Nasr and Erakey 1967, Aboul-Nasr and Erakey 1968a, Weeks et al. 2010). Continued research to better understand the role of exogenous factors in host location will play an important role in the commercialization of traps that utilize attractants to improve trap efficacy.

Upon locating their host bed bugs position themselves using their tarsal claws to gain leverage (Usinger 1966) and insert their flexible stylet through the skin and begin probing until locating a blood vessel upon which they will begin to feed (Lavoipierre

1965, Usinger 1966). The feeding time and amount of blood consumed vary based upon the developmental stage and gender (Marshall 1981, Reinhardt and Siva-Jothy 2007, Suchy and Lewis 2011, Barbarin et al. 2013, Pereira et al. 2013). In order to become fully engorged, bed bugs feed up to10 minutes (Pereira et al. 2013). Nymphs typically consume 3-6 times, males ~1.5 times, and females 2 times their own body weight (Reinhardt and Siva-Jothy 2007, Lehnert 2013). Adult females probably feed in larger quantities than males due to their larger size and nutritional demand to produce eggs compared to sperm in males (Araujo et al. 2009). Bed bugs are reported to acquire a blood meal approximately every 7 days (Reinhardt and Siva Jothy 2007) but may feed more frequently depending upon environmental conditions (Reinhardt et al. 2010) or when food is continuously available as would be expected under field conditions (Pereira et al. 2013).

Following the completion of their blood meal, bed bugs return to their harborage, where they remain until the blood meal is digested (Usinger 1966, Mellanby 1932, 1939). The recently fed nymphs will utilize the blood meal to develop into the next nymphal instar, or in the case of fifth instar, into adulthood (Usinger 1966). In the adult stage, females are mated shortly after obtaining their blood meal, while still engorged (Usinger 1966). Females are unable to defend themselves from sexual advances by males while in an engorged state, compared to unfed females that enter a “refusal posture” preventing penetration by the advancing male(s) (Morrow and Arnqvist 2003, Siva-Jothy 2006, Reinhardt et al. 2009a).

Copulation in bed bugs occurs via traumatic insemination, in which the male pierces the abdominal wall of the female with their intromittent organ (paramere), in a specialized region (ectospermalege) of the abdomen. The ectospermalege acts as a physical guide for insertion of the paramere, directing the injection of the sperm and accessory gland fluids into another specialized organ (mesospermalege) located directly beneath the ectospermalege (Carayon 1966). For a complete understanding of the reproductive physiology of bed bugs refer to Carayon (1966) and Reinhardt and Siva Jothy (2007).

Adult females are mated by males at a rate of 20-25 times higher than what is needed to maintain fecundity which is costly to females, resulting in reduced longevity and reproductive success (Stutt and Siva-Jothy 2001, Morrow and Arnqvist 2003, Reinhardt et al. 2003, Polanco et al. 2011b) and beneficial to males by last-male sperm precedence (Stutt and Siva-Jothy 2001). Stutt and Siva-Jothy (2001) suggested that multiple traumatic insemination is a coercive copulatory strategy imposed by the male, resulting in a sexual conflict of interests. For an in depth discussion of sexual conflict in bed bugs refer to Stutt and Siva-Jothy 2001, Morrow and Arnqvist 2003, Reinhardt and Siva-Jothy 2007, Pfiester et al. 2009a, Lange et al. 2013, and Reinhardt et al. 2014.

Behavioral Ecology

Bed bugs spend most of their lives in refugia living in aggregations that contain bed bugs of all stages, feeding status and mating status (Johnson 1941, 1942, Usinger 1966, Reinhardt and Siva-Jothy 2007, Pfiester et al. 2009b). This aggregate lifestyle provides a number of potential benefits such as reduced water loss, increased mating opportunities and protection from predators (Benoit et al. 2007, Siljander et al. 2008, Benoit 2011). Pheromones, in conjunction with thigmotactic behavior, play a key role in maintaining bed bug aggregations (Marx 1955, Usinger 1966, Levinson and Bar Ilan

1971, Siljander et al. 2007, 2008, Olsen et al. 2009, Weeks et al. 2011, Gries et al. 2014). The aggregation pheromone consists of two components, a volatile one that guides bed bugs back to the aggregation, and a less volatile one that causes an arrestant behavior once they are back in the aggregation (Gries et al. 2014).

In addition to using pheromones to maintain aggregation behavior, bed bugs also release a volatile alarm pheromone, causing bed bugs to disperse from potential threats (Levinson and Bar Ilan 1971). Interestingly, the two major constituents, (*E*)-2-hexenal and (*E*)-2-octenal, of the alarm pheromone are also found in the aggregation pheromone and can elicit either an aggregation or alarm behavior depending upon their concentration in the pheromone that is being released (Siljander et al. 2008). While alarm pheromones are most often intended to protect against external threats (predators), in bed bugs they are also used for intra-specific communication, to defend against mate-seeking males (Ryne 2009, Harraca et al. 2010). Males rapidly mount and attempt to mate with any large, newly fed, individual that is nearby regardless of sexual maturity or gender (Rivnay 1933, Rao 1972, Ryne 2009). Attempted mating by adult males, results in the release of alarm pheromone by the male being mounted (Ryne 2009), while late instar nymphs release a nymph-specific alarm pheromone to fend off the unwanted mating attempt (Harraca et al. 2010).

Reinhardt and Siva-Jothy (20070 noted that population and dispersal ecology is an area where perhaps the least progress has been made since the early works of Mellanby (1939), Johnson (1941) and Usinger (1966). The composition of a bed bug population varies widely within the published literature, with adults making up anywhere from 5-32% of the population depending upon the study. Early field studies conducted under a variety of environmental conditions reported adult males comprising 18-32% of the population based upon trap catch (Mellanby 1939, Johnson 1941). Newberry and Jansen (1986) collected and counted bed bugs from sheets placed out on the floor of infested huts that were fumigated and found the adult male proportion ranged from 5.6-30%. More recently Wang et al. (2010) reported adults making up 22% of bugs captured in pitfall traps placed out in 20 infested apartments. In another study, using sticky traps baited with CO_2, heat and a synthetic pheromone, adults made up a much smaller proportion (5-7%) of the trapped bed bugs (Schaafsma et al. 2012). A limitation of all of these studies is that they are based upon severe infestations and involve a limited number of replicates.

Within a bed bug population adult males and females exist in a 1:1 ratio (Johnson 1941, Newberry and Jansen 1986, Stutt and Siva-Jothy 2001). However, Pfiester et al. (2009b) suggested that adult sex ratios at the aggregation-level may be different than at the population-level. Under laboratory conditions, Pfiester et al. (2009b) demonstrated that adult females formed aggregations with fewer adult males as population density increased, a finding that was confirmed by Naylor (2012) using an elaborate arena intended to simulate field conditions. Lehnert (2013) also observed female-dominated aggregations, under field conditions. It has been suggested that variations in adult sex ratio at the aggregation level could be a female strategy to avoid traumatic insemination (Weeks 2011, Lehnert 2013). When the density of males inside aggregation increases, females move away from aggregations. This movement of adult females away from aggregations, may be an indication that they are the primary dispersal stage (Pfiester et al.

2009b, Weeks 2011). Whether either of these explanations are true remains speculative and requires further investigation.

Within an infested dwelling, the majority of bed bugs are believed to be associated with furniture used by the host for sleep or rest. In a field study of 13 infested apartments, Potter et al. (2006) found over 90% of bed bugs hiding at beds (70%) and upholstered furniture (23%). However, their findings are based upon counting bed bugs that they were able to locate during visual inspection and thus may be skewed due to the ease of locating bugs on the furniture compared to less predictable areas off furniture. Wang et al. (2010) placed pitfall-style traps under the legs of beds and furniture and found 89% of the trapped were in the outer well of the trap, suggesting that of the trapped bed bugs, the majority were not on the furniture and would be easily missed during visual inspection. In the same study, bed bugs were also captured in pitfall-style traps placed in hallways just outside of the infested apartment's entry door, demonstrating the presence of bed bugs far from host sleeping areas and providing the first real evidence of active dispersal of bed bugs between apartments (Wang et al. 2010). Booth et al. (2012) and Saenz et al. (2012) provided additional genetic evidence in support of active dispersal of bed bugs between apartments, however definitive proof is still lacking. Bed bug activity in areas away from host sleeping and resting areas has important implications for pest management professionals in regards to the proper assessment and treatment of bed bug infestations.

Movement of bed bugs away from aggregations can lead to a number of negative outcomes, including increased risk of mortality from predation or desiccation and decreased ability to relocate hosts and future mates (Benoit et al. 2011). Thus movement away from the safety provided by harborage sites, in theory, should have benefits that outweigh the costs of remaining within the aggregation (Pulliam & Caraco 1984, Wertheim et al. 2005). Pfiester et al. (2009b) suggested that adult females were the most likely, and young nymphs the least likely, stages to disperse from aggregations. How and Lee (2010) supported the findings of Pfeiester et al. (2009) in a laboratory study with the tropical bed bug. In their study, they demonstrated 5th instar nymphs and adults of both sexes dispersed farther than younger nymphs and that starved bed bugs traveled farther than fed bed bugs, with the exception of recently fed adult females which were more active and traveled farther than other stages regardless of feeding status. The exact triggers for the dispersal of bed bugs away from aggregations are not known but may include changes in population density (Wertheim et al. 2005, Naylor 2012), temperature (Bell 1990), repellency from insecticides (Romero et al. 2009a), host availability (Romero et al. 2010a, Suchy and Lewis 2011), avoidance of traumatic insemination by males (Stutt and Siva-Jothy 2001, Pfiester et al. 2009a,b, How and Lee 2011, Lehnert 2013) and location of additional host feeding sites in other rooms within a dwelling or between living units in multi-occupancy dwellings (How and Lee 2010).

Medical and Social Relevance

The negative impact of bed bugs upon their human host can be manifested clinically (Goddard and deShazo 2009, Doggett et al. 2012), socially (Rossi and Jennings 2010, Eddy et al. 2011, Aultman 2012, Susser et al. 2012), and economically (Rossi and Jennings 2010, Eddy et al. 2011, Aultman 2012, Doggett et al. 2012, Wong et al. 2012). Additionally, improper methods associated with eradication efforts of infestations can result in hazards to human health and/or structure in relationship (Pinto et al. 2007, Centers for Disease Control and Prevention 2011, Doggett et al. 2012).

Given their obligatory blood-feeding behavior, their preference for human hosts, and the many different disease pathogens which they carry (Ryckman et al. 1981), bed bugs would appear to be excellent agents for disease transmission to humans. However to date, bed bugs have not been shown to be an effective disease vector (Goddard and deShazo 2009, Doggett et al. 2012). Transmission of pathogens by bed bugs has been demonstrated, but only under laboratory conditions. For example, Blow et al. (2015) demonstrated mechanical transmission of Hepatitis B, under experimental conditions. More recently, Salazar et al. (2015), demonstrated the ability of bed bugs to acquire *Trypanasoma cruzi,* the pathogen responsible for Chagas disease, as a result of feeding upon infected mice under laboratory conditions. In a separate laboratory experiment they demonstrated the transmission of *T. cruzi* to previously uninfected mice after 30 days of cohabitation with bed bugs that were artificially infected with *T. cruzi* before being placed in with the mice (Salazar et al. 2015). While experimentally manipulated laboratory studies continue to raise concerns, vector competency of bed bugs under natural field conditions remains unproven. In a review of the published literature, Goddard and deShazo (2012) concluded that although transmission of over 40 diseases have been attributed to them, little evidence exists to support human disease transmission by bed bugs. Still, researchers warn that the threat posed by bed bugs to vector and spread diseases to humans should be taken seriously particularly in areas where the presence of disease pathogens, bed bugs and humans overlap in abundance (Lowe and Romney 2011, Leulmi et al. 2015, Salazar et al. 2015) or where bird/bat populations roosting in man-made structures may potentially support undiscovered enzootic transmission cycles (Adelman et al. 2013). Given the significance of this topic, the subject of bed bugs as disease vectors will no doubt continue to be investigated.

Despite the fact that bed bugs are not considered medically important vectors of disease, they are still be considered a pest of public health importance. Reactions to proteins in the saliva of bed bugs can cause a wide range of reactions among those who are bitten ranging from no reaction, to localized swelling, intense itching, scarring of tissue, and in rare cases anaphylactic shock (Parsons 1955, Ter Poorten and Prose 2005, Goddard and deShazo 2009, Doggett et al. 2012). In very severe infestations there is also a concern that iron deficiency or anemia may result from the blood loss caused by the feeding activity of bed bugs (Venkatachalam and Belavady 1962, Pritchard and Hwang 2009, Doggett et al. 2012). Secondary infections can also occur in association with the scratching of bed bug bites (Millikan 1993, Goddard and deShazo 2009).

The presence of bed bugs, or even the thought that they are present, can cause anxiety, insomnia, and nightmares (Hwang et al. 2005, Doggett et al. 2012) as well as a wide variety of mental health effects (Goddard and deShazo 2009, 2012, Doggett et al. 2012, Susser et al. 2012). People suffering from bed bug infestations are often ostracized which can lead to social isolation (Davies et al. 2012) and have been denied access to employment offices, healthcare clinics, libraries and other public services (Eddy and Jones 2011, Aultman 2012). In an effort to rid infestations, misuse of pesticides, particularly by consumers can lead to excessive exposure, harm and even death (Center for Disease Control and Prevention 2011). Homes have been set ablaze during structural heat treatments performed by homeowners as well as professionals, and widespread use of rubbing alcohol and other combustible products pose a serious health and fire risk (Doggett et al. 2012).

Direct costs associated with the hiring of a pest control firm to eliminate bed bug infestations is costly and often more expensive than many can afford, especially among those in underserved communities where bed bugs are most prevalent (Pinto et al. 2007, Davies et al. 2011, Wang and Cooper 2011, Doggett et al. 2012, Wong et al. 2013). However, the economic costs associated with bed bugs extend beyond those associated with the hiring of a pest management professional and may include discarding of furniture and personal belongings, loss of wages, and medical costs (Doggett et al. 2012). Additionally, businesses affected by bed bugs or even suspected to have been affected by as much as a single bed bug, can suffer loss of business, damage to reputation, and potentially costly litigation (Pinto et al. 2007, Doggett 2012)

Detection of Bed Bugs

Detection of bed bugs is important not only for the initial identification of infestations, but also to evaluate the effectiveness of treatments, and to determine when infestations have been eliminated. Thus the ability to detect bed bugs when they are present only in small numbers, is critical in managing the costs associated with the elimination of infestations (Pinto et al. 2007, Wang and Cooper 2011) and preventing the continued spread of bed bugs (Pinto et al. 2007, Doggett et al. 2012, Vaidyanathan and Feldlaufer 2013). Unfortunately, the cryptic nature and secretive lifestyle of bed bugs make their detection difficult when their numbers are small (Pinto et al. 2007, Wang et al. 2009, 2010, 2011, Wang and Cooper 2011). Surprisingly, even when bed bug infestations are well established they often go unreported. In a study by Wang et al. (2010), 50% of residents in apartments with bed bug infestations indicated they were unaware the bugs were present. One reason this occurs is that not everyone develops bite symptoms after being bitten (Reinhardt and Siva-Jothy 2009b, Potter et al. 2010). Even when infestations are known to occupants of infested dwellings, they may go unreported because the occupants are embarrassed, fear some type of negative consequence, or are trying to avoid having people enter their home for inspection or treatment (Pinto et al. 2007, Vaidyanathan and Feldlaufer 2013). Without pro-active inspection programs, infestations that go unreported, can promote further spread of bed bugs within living communities and out into society and are more expensive to eliminate when they are eventually identified.

Visual inspection of beds and upholstered furniture provides immediate results but can be labor intensive and has been found to be unreliable for detection of bed bugs present in low numbers (Wang et al. 2010, 2011). Sticky traps are used by 44% of pest management professionals (Potter et al. 2015), largely because of pest management professionals' familiarity with this type of trap for cockroaches, and their inexpensive cost. However, detection using unbaited sticky traps along with harborage style traps (i.e. corrugated cardboard or crevice-based traps) have not proven effective, are inconsistent, and are unreliable for detection of low-level bed bug activity (Potter 2005, Harlan 2007, Harlan et al. 2008, C. Wang, unpublished data,)

Pitfall-style interception devices were used by Mellanby (1939) and Johnson (1941) to study the behavioral ecology of bed bugs. More recently, a variety of passive (without lure) and active (contain one or more lures) pitfall-style monitoring devices have been developed for commercial use. Active monitors use CO_2, heat, chemicals, or a combination of one or more of these to attract bed bugs (Pinto et al. 2007, Wang et al. 2009, 20011, Anderson et al. 2011, Singh et al. 2013a, Singh et al. 2015a). Unfortunately, most of these traps are expensive, can be logistically difficult to deploy, and vary in effectiveness (Wang et al. 2011, Vaidyanathan and Feldlaufer 2013, Singh et al. 2015a). Singh et al. (2015a) suggested that the low CO_2 rates (< 50 ml/min) released by most commercial monitors are too low to be competitive with the human respiration rate (250 ml/min) making them ineffective or limiting their effective range. Due to high cost, along with the other limitations, most of the active monitors are no longer commercially available. Singh et al. (2015a) developed an effective and economically affordable (~$19) active monitor that uses sugar, yeast, and water to produce CO_2 at a release rate of 405 ml/min for over 8 hours, providing consumers with a very effective "do-it-yourself" option for detecting bed bugs. However, the cumbersome nature of the trap makes it impractical for use by professionals.

Passive pitfall-style interception devices placed under the legs of beds and upholstered furniture for 7 days, have been shown to be very effective in the detection of bed bugs present even in low numbers (Wang et al. 2011). Passive interceptor traps placed under the legs of beds and upholstered furniture, benefit from the sleeping host which serves as a lure. Interestingly, placement of passive pitfall-style interceptors away from host sleeping areas has also been shown to be effective in capturing bed bugs (Wang et al. 2010). The biggest disadvantage of using traps to detect bed bug activity is that two visits are required, one to place traps out and a second visit to inspect the devices for trap catch, resulting in a delay in detection results and increased labor costs.

The use of canines for the detection of bed bugs has become increasingly popular (Cooper et al. 2007, Pinto et al. 2007, Potter et al. 2015). Like visual inspection, canine scent detection offers immediate results but is more efficient, and does not require beds and other furniture to be flipped, as is typically the case with visual inspections. Moreover, because bed bug sniffing dogs rely on their olfactory senses rather than sight for detection, bed bugs that are not visually accessible can still be detected. The use of dogs to detect bed bugs, is particularly well suited for large scale inspections and inspection of non-traditional environments, such as movie theaters, retail stores, schools, and other areas where visual inspection or placement of traps may be economically impractical (Pinto et al. 2007). The possibility of false positive alerts (indicating that bed bugs are present when they are not) is a major drawback associated with canine scent detection.

Only one study has been conducted to date to evaluate canines in the detection of bed bug (Pfiester et al. 2008). The study was a controlled field study, using planted hides in hotel guest rooms. The dogs detected 98% of the hides with bed bugs and had no false indications on hides not containing bed bugs (Pfiester et al. 2008). The hotel room study clearly demonstrated the high level of accuracy of trained canines in detecting bed bugs under controlled conditions using well trained dogs and highly skilled dog handlers. Whether the results of the Pfiester et al. (2008) translate to inspections conducted by commercially available canine scent detection teams, under naturally occurring conditions is unclear (Cooper 2007a, Pinto et al. 2007).

Currently, there is no one detection method that can be relied upon for detection of low-level bed bug activity. A combination of at least two methods, such as visual inspection plus placement of traps, or canine detection coupled with confirmation by visual inspection and/or placement of traps is recommended (Vaidyanathan and Feldlaufer 2013). Continued research regarding the factors that influence bed bug orientation to hosts, and physical objects will play an important role in the development of new detection tools/methods that are economically affordable (Singh et al. 2015b). In spite of published studies demonstrating the importance of detecting bed bugs, the pest management industry continues to rely on methods that are not reliable for detecting bed bugs present in small numbers. Among 236 pest management professionals interviewed, 100% reported using visual inspection to identify infestations while only 56% report using active or passive pitfall-style monitors which have been shown to be the most reliable tool for low-level detection of bed bugs (Potter et al. 2015). Additionally of those surveyed 44% reported using sticky traps, and 42% employ the use of bed bug sniffing dogs.

Control Tools and Methods

Bed bugs remain the most difficult pest facing today's pest management professional (Pinto et al. 2007, Doggett et al. 2012, Potter et al. 2015). Early detection can be difficult, the ability of trained professionals varies widely (Doggett et al. 2012), bed bug management tools vary in effectiveness; with complete lack of proven efficacy for some (Jones and Bryant 2012, Singh et al. 2014), cooperation is essential but not always received (Stedfast and Miller 2014, Wang et al. 2014), and the costs associated with elimination of infestations can be high (Pinto et al. 2007, Wong et al. 2013, Stedfast and Miller 2014). Stephen Doggett (Department of Medical Entomology, ICPMR, Westmead Hospital, Australia) was among the first to recognize the complexity of the problem associated with the delivery of a consistently effective approach to bed bug management. He addressed this issue by creating a Code of Practice for the control of bed bug infestations in Australia (Code of Practice 2006). The Code of Practice provided the most comprehensive recommendations for bed bug control at that time, and is now in its fourth edition. Since its inception, the Australian Code of Practice has been unique, providing recommendations for the use of specific products for the detection and control of bed bugs based upon the efficacy. In 2011, a similar Code of Practice was produced in Europe (Madge 2011), and in the United States, the National Pest Management Association (NPMA) published Best Management Practices (BMPs) for bed bugs (NPMA 2011). While avoiding the recommendation of specific products, the BMPs do provide a set of recommended practices for pest management professionals to follow, and for consumers to refer to, when selecting a professional pest management firm for the control of bed bugs.

Resistance in bed bugs to a wide variety of pesticides is well documented (Zhu et al. 2010, Davies et al. 2012, Doggett et al. 2012, Koganemaru et al. 2013a, Gordon et al. 2014). However, resistance to pesticides among bed bugs is not a new phenomenon. Widespread resistance to DDT had become widespread among bed bugs by 1947 (Usinger 1966). By 1956, the National Pest Control Association, issued the recommendation to use the organophosphate, malathion, to combat DDT resistant bed bugs. Most pesticides previously used for controlling bed bugs are no longer available and have been replaced by other less toxic and less persistent chemistries. Currently, synthetic pyerthroids are the most commonly used class of insecticides for the control of urban pests including bed bugs. However, widespread resistance in bed bugs to pyrethroids has been documented both in the United States (Romero et al. 2007b, Yoon et al. 2008, Zhu et al. 2010, 2013) and other parts of the world (Lilly et al. 2009, Kilpinen et al. 2011, Tawatsin et al. 2011).

Few alternative classes of chemistry exist that are available for control of urban pests, and even fewer are registered for use on bed bugs (Gordon et al. 2014). One alternative product, Phantom® which contains the active ingredient chlorfenapyr (chemical class: pyrrole), was shown to be effective at killing pyrethroid-resistant bed bugs but is slow acting (Romero et al. 2010b). More recently, "dual action" products, containing active ingredients from two different classes of chemistry (synthetic pyrethroid and neonicotinoids) with different modes of action, have been developed as a solution for treating pyrethroid-resistant bed bugs. Potter et al. (2012) demonstrated the effectiveness of dual action products in the lab as well as in the field, but cautioned that development of resistance to these products remains a concern, a concern which proved to be true when resistance to the combination insecticides was identified in field collected populations of bed bugs (Gordon et al. 2014). Bed bugs employ a variety of resistance mechanisms to combat the toxic effects of pesticides (Adelman et al. 2011, Mamidala et al. 2012, Koganemaru et al. 2013b, Zhu et al. 2013). A recent study of 24 bed bug populations showed that 70% of the populations tested, possessed multiple mechanisms of resistance, including reduced cuticular penetration, knock-down resistance (kdr), increased activity of detoxification enzymes (cytochrome P450s), and ATP-binding protein transporters (ABC transporters) (Zhu et al. 2013). Based upon these findings, the rapid development of resistance by bed bugs to new products is not surprising and is likely to continue to be a challenge in the future.

Interestingly, pesticide dust formulations have proven to be more effective against bed bugs than liquid residuals containing the same active ingredient (Romero et al. 2009b). Pyrethroid-resistant bed bugs suffered observed 90-100% mortality when exposed to pesticide dusts containing a pyrethroid as the active ingredient (Romero et al. 2009b). Why dusts containing a pyrethroid are effective against bed bugs with high levels of pyrethroid resistance is unclear. Desiccant dusts containing either diatomaceous earth (DE) or Silicates (silica gel) have also been shown to be effective against bed bugs, with silicates being the more effective of the two (Benoit 2009, Romero et al. 2009b, Potter et al. 2014). One product in particular, CimeXa® dust, has been shown to be highly effective against bed bugs in very small quantities under both laboratory as well as field conditions (Potter et al. 2014). Our lab also found CimeXa to be the most effective dust among eight insecticide dusts evaluated, causing 100% bed bug mortality in three separate experiments using various exposure methods (Singh et al., unpublished data). We also demonstrated a horizontal transfer effect in which bed bugs not exposed to CimeXa dust were killed by contacting bed bugs that were exposed to CimeXa dust. (Singh et al., unpublished data). Whether bed bugs will be able to develop resistance to the desiccant dusts remains to be seen.

No one product is effective for the elimination of well-established infestations. Instead an integrated pest management approach (IPM) is necessary for the elimination of infestations and to reduce further spread of bed bugs (Pinto et al. 2007, Wang and Cooper 2011, Doggett et al. 2012, Koganemaru et al. 2013a). Non-chemical tools and methods have proven very effective (Potter et al. 2007, Wang and Cooper 2011, Doggett et al. 2012). While non-chemical methods are gaining acceptance, their adoption by pest management professionals continues to be slow. According to pest management professionals surveyed in 2014, 95% indicated that they still normally use pesticides to control bed bugs, among which 97% typically treat the bed (Potter et al. 2015)

Encasement of beds as a bed bug management measure has been widely adopted by the pest management industry, with 84% of surveyed professionals indicating that they use encasements in their control programs (Potter et al. 2015). Encasement of mattress and box springs, makes it unnecessary to discard infested beds, immediately reduces bed bug populations, and increases the efficiency of follow-up inspections (Cooper 2007b, Wang and Cooper 2011, Koganemaru and Miller 2013a). Vacuums and steam machines can be used effectively to quickly eliminate bed bugs aggregating on beds and furniture or any other place where bed bugs are visibly present. While effective, vacuums fail to pull bed bugs out of cracks and crevices, and often fail to dislodge eggs that are glued to the substrate (Pinto et al. 2007, Potter et al. 2007). The use of steam, on the other hand, provides penetration into cracks as well as through fabric on mattresses, box springs, and upholstered furniture (Pinto et al. 2007, Potter et al. 2007, Puckett et al. 2013). Among professionals surveyed in 2014, only 62% of professionals reported using vacuums, and even fewer (48%) use steam in their bed bug management programs (Potter et al. 2015).

In addition to using steam to kill bed bugs, the use of heat has been exploited in a number of ways for the control of bed bugs, including; 1) hot laundering of bed linens clothing and other items that can be exposed to a hot cycle in a washing machine or dryer (Kells 2006, Naylor and Boase 2010, Wang and Cooper 2011, Haynes and Potter 2013), 2) containerized heat for heat-treatment of infested items (Pinto et al. 2007, Pereira et al.

2009), and 3) whole structure heat treatments to eliminate infestations (Pinto et al. 2007, Getty et al. 2008, Pereira et al. 2009, 2011). Kells and Goblirsch (2011) demonstrated temperatures of 48.3 ºC and 54.8 ºC were necessary to provide immediate mortality of adults and eggs, respectively, while temperatures below these lethal limits can still result in mortality if held for a sufficient amount of time. Exposure to extreme cold is also an effective method for killing both bed bugs and their eggs but has not been used as widely as exposure to heat. Infested items placed in a freezer for 4 days at −17.8 ºC will destroy bed bugs and their eggs (Wang and Cooper 2011, Olson et al. 2013)

Other non-chemical methods involve simplifying the environment. For example, using a simple metal bed frame to get a mattress off of a floor or to replace a heavy wooden bed frame that is hard to move and difficult to inspect or treat (Wang et al. 2014). Isolating beds by moving them away from the wall, tucking in sheets, and placing pitfall-style interceptors under the legs of the bed can reduce the number of bed bugs and play an important role in a management program (Pinto et al. 2007, Wang and Cooper 2011). Eliminating clutter, particularly in areas close to sleeping and resting areas is another important measure that can help in the elimination of infestations, along with the disposal of infested items and furniture that are no longer wanted (Pinto et al. 2007, Potter et al. 2007, Wang and Cooper 2011, Doggett et al. 2012, Koganemaru and Miller 2013a). Proper building maintenance, changes in room design and type of furnishings can make environments less bed bug friendly but are beyond the scope of this dissertation.

Current Challenges in the Control of Bed Bugs

Bed bugs are currently recognized as the most difficult pest to control by pest management professionals in the United States (Potter et al. 2013a) and continue to pose significant social, economic, and public health concerns (Susser et al. 2012, Doggett et al. 2012, Aultman 2013, Koganemaru and Miller 2013a, Wong et al. 2013). In a recent review article by Doggett et al. (2012) the authors stated "Indications are that bed bugs will continue be a societal pest for many years to come. In the near future, there is unlikely to be any magical silver-bullet technology developed for controlling this pest which might rapidly defeat this insect, as in the case of DDT during the 1950s. This means that people will continue to be exposed to bed bugs and all their various deleterious effects." The authors concluded by saying "As of late 2011, it is not possible to write a definitive conclusion to this story; the global fight against bed bugs has only just begun." Koganemaru and Miller (2013a) also suggested that the number of bed bug infestations will continue to increase worldwide because we lack effective methods that are low in cost. These opinions from leading scientists in the field of bed bug biology and control paint a bleak future in relationship to our society-wide battle against bed bugs.

Survey results support the assertion that the impact of bed bugs upon society continues to increase. Among 236 pest management companies surveyed, 99% indicated that their company had provided services for bed bugs, 75% reported an increase in their bed bug service work in 2014, and 64% said they believed infestations were continuing to increase in their part of the country (Potter et al. 2015). The survey also showed an increase from 2010 to 2014, in the percentage of pest management firms treating bed bugs in non-residential settings such as schools and day cares (33% increase), office buildings (27% increase), and public transportation (20% increase).

While there have been significant advances in the tools and methods available for the control of bed bugs (Pinto et al. 2007, Wang and Cooper 2011, Doggett et al. 2012, Haynes and Potter 2013, Koganemaru and Miller 2013a), the cost of control remains high and is a financial burden in low-income communities (Doggett et al. 2012, Koganemaru and Miller 2013a, Wong et al. 2013). Potter et al. (2015) reported the median cost for treatment of single family home to be $1,225, and the average cost for treatment of a single apartment ~ $500 (Stedfast and Miller 2014). In apartment communities with high infestation rates, the cost of bed bug control can have far exceed costs budgeted for entire pest control and can be financially devastating (Wong et al. 2013, Stedfast and Miller 2014). Twenty six housing authorities surveyed in Virginia spent $404,364 over a six month period to treat 1,047 apartments, with $160,000 of the money being spent by a single housing authority in Richmond (Wong et al. 2013). Unfortunately, low-income communities are suffering disproportionately higher infestation rates than the rest of the society (Wang et al. 2008, Robinson and Boase 2011, Wong et al. 2013, Stedfast and Miller 2014) and now serve as a reservoir for the continued spread of bed bugs in residential communities and into the surrounding society (Robinson and Boase 2011, Doggett et al. 2012, Vaidyanathan and Feldlaufer 2013). The increasing prevalence of bed bugs in both residential and non-residential settings throughout the country (Potter et al. 2013a) is a clear indicator that efforts to limit the spread of bed bugs have failed.

In spite of the development of effective tools for the detection and control of bed bugs, inadequate pest control continues to be a problem. The pest management industry continues to rely on pesticides in their bed bug management programs due to the low cost and ease of application, despite the documented problem of pesticide resistance (Potter et al. 2015). The best management practices created by the National Pest Management Association recommend an integrated pest management approach utilizing a combination of both chemical and non-chemical methods; however the tools and methods recommended are not widely used by the professional pest management industry (Potter et al. 2015).

Lack of cooperation by both residents and property management can complicate bed bug control efforts resulting in increased costs and time to eliminate infestations (Pinto et al. 2007, Wang et al. 2007, Potter et al. 2012, Singh et al. 2013b, Wang et al. 2013). Some examples include, refusing entry for inspection or treatment, refusal to eliminate clutter or launder infested bed linens. There are many reasons residents fail to cooperate. In some cases they simply don't care or have become apathetic after repeated failed attempts by property management and pest management professionals. However, economic, physical, or mental barriers can also exist that prevent individuals from carrying out the necessary activities. In such cases without intervention from a family friend or relative, home aide, property management, or the pest management professional, infestations may not be eliminated.

A lack of methodology for determining when infestations have been eliminated can cause the premature termination of treatment efforts, which in turn can lead to chronic infestations, and promote further spread of bed bugs within housing complexes and into the community (Wang and Cooper 2011). Numerous field studies have reported bed bug reduction of 90% or more in treated apartments but have failed to completely eradicate the infestations (Wang et al. 2009, Moore and Miller 2009, Wang et al. 2010, Potter et al. 2012, Singh et al. 2013b). Pest management professionals typically terminate treatments once bed bugs are no longer observed visually or when residents report being satisfied with the results. However, when populations are reduced to a low-level, detection can be difficult and easily missed during inspections (Pinto et al. 2007, Wang and Cooper 2011, Vaidyanathan and Feldlaufer 2013). In a field study by Wang et al. (2009) bed bugs were found in pitfall-style interceptor traps in apartments believed eradicated, based upon visual inspection. Moreover, at the conclusion of their study, residents' living in units where bed bugs still existed indicated that they were not being bitten. The premature termination of bed bug treatments can potentially lead to population rebound and movement of bed bugs to previously un-infested locations.

Few property managers take a proactive approach to bed bug management, instead most are reactionary, only treating infestations that have been reported. However, many infestations go unreported by residents. In turn, unreported infestations lead to the escalating infestation rates being seen in many multifamily housing communities (Pinto et al. 2007, Doggett and Russell 2008, Wang and Cooper 2011, Wong et al. 2013, Stedfast and Miller 2014). There is a great need for education of residents regarding the signs and symptoms of bed bugs and the importance of reporting suspected infestations. Likewise, property managers must encourage reporting of infestations by residents, assuring them that there will be no negative consequences associated with alerting property management of the problem. Finally, property managers must be educated on the importance of taking a proactive approach to identify infestations rather than relying on reporting by residents.

Robinson and Boase (2011) predict the gradual disappearance of bed bugs from middle and high socio-economic sectors, suggesting the rate of appearance of new infestations will fall rapidly, leading to a flattening out, followed by decline of the resurgence in this sector. In contrast they, like others (Pinto et al. 2007, Wang and

Cooper 2011, Doggett et al. 2012, Koganemaru and Miller 2013a), went on to predict that the low-income sector of society will be unable to afford technology-intensive treatment methods. Thus in order to stem the spread of bed bugs throughout society, it will be necessary to create programs that are effective, sustainable, and economically practical, in low-income communities (Robinson and Boase 2011, Wang and Cooper 2011, Doggett et al. 2012, Koganemaru and Miller 2013a). While the development of cost-effective bed bug management strategies is critical, unless it is embraced and adopted on a wide-scale basis by pest management professionals, city authorities, and housing managers, the advancements will serve little value in altering the course of the bed bug resurgence.

CHAPTER TWO

Accuracy of Trained Canines for Detecting Bed Bugs

Abstract

Detection of low-level bed bug, *Cimex lectularius* L. (Hemiptera: Cimicidae), infestations is essential for early intervention, confirming eradication of infestations, and reducing the spread of bed bugs. Despite the importance of detection, few effective tools and methods exist for detecting low numbers of bed bugs. Scent dogs were developed as a tool for detecting bed bugs in recent years. However, there are no data demonstrating the reliability of trained canines under natural field conditions. We evaluated the accuracy of 11 canine detection teams in naturally infested apartments. All handlers believed their dogs could detect infestations at a very high rate (≥ 95%). In three separate experiments, the mean (min, max) detection rate was 44 (10-100)% and mean false positive rate was 15 (0-57)%. The false positive rate was positively correlated with the detection rate. The probability of a bed bug infestation being detected by trained canines was not associated with the level of bed bug infestations. Four canine detection teams evaluated on multiple days were inconsistent in their ability to detect bed bugs and exhibited significant variance in accuracy of detection between inspections on different days. There was no significant relationship between the team's experience or certification status of teams and the detection rates. These data suggest that more research is needed to understand factors affecting the accuracy of canine teams for bed bug detection in naturally infested apartments.

Introduction

Bed bugs have plagued mankind since the beginning of recorded history (Potter 2011, Davies et al. 2012). Although once prevalent in the U.S. and other developed countries, they were virtually eradicated in many parts of the world shortly after World War II through the widespread use of pesticides such as DDT and malathion (Pinto et al. 2007). After a nearly 50 y absence, beginning in the late 1990s, bed bugs returned in an unexpected and dramatic fashion, sweeping across North America, the United Kingdom, and Australia (Doggett et al. 2011, Eddy and Jones 2011, Davies et al. 2012). The global resurgence of bed bugs has prompted research on a wide variety of topics including basic biology, behavior, physiology, chemical ecology, management practices, and methods of detection (Davies et al. 2012). Early detection of bed bugs is recognized as a key factor in reducing both the costs associated with bed bug management and the spread of bed bugs from infested dwellings to new locations (Pinto et al. 2007). Despite the importance of detection, effective tools and methods for identifying low-level populations remain limited (Wang et al. 2011, Wang and Cooper 2012, Lewis et al. 2013).

Current methods of detection include visual inspection, deployment of monitoring devices, and canine scent detection. Visual inspection, the most common detection method employed, is labor intensive and very intrusive, requiring beds and furniture to be flipped over for inspection. Moreover, because bed bugs are so secretive, visual inspections are not regarded as a reliable method of detection when only a few bugs or eggs are present (Cooper 2007a, Pinto et al. 2007). Wang et al. (2011) compared the effectiveness of visual inspection, passive pitfall-style interceptors and active (with lure) monitors in lightly infested apartments. In their study, when very few bugs were present, the greatest number of infestations was detected by passive pitfall style monitors (70%) placed under the legs of beds and upholstered furniture for 7 d, compared to visual inspections (50%) and various active monitors (10 - 60%) placed next to beds and upholstered furniture for 1 d. Detection rates using interceptors can be increased to 90% or greater by increasing the trapping interval from 7 to 14 d (Cooper, unpublished data). The limitation of using monitoring devices is that they do not provide immediate results, and a minimum of two visits are required.

Due to the limitations associated with visual inspection and monitoring devices, the use of trained dogs has gained popularity as an alternative method for identifying bed bug infestations (Cooper 2007a, Pinto et al. 2007, Potter et al. 2011). This method could be efficient for large area inspections and provides immediate results, a combination not available with other detection tools and methods. Canine scent detection is especially well suited for less traditional settings such as schools, office buildings, retail stores, and movie theaters, where other detection methods are not aesthetically or economically practical (Pinto et al. 2007, Wang and Cooper 2011). However, their effectiveness in correctly identifying natural infestations has never been reported.

Numerous studies have demonstrated the effectiveness of trained dogs for the detection of biological and non-biological odors (Johnston 1999, Browne et al. 2006), including a number of insect pests such as gypsy moths (Wallner and Ellis 1976), screwworm pupae and larvae (Welch 1990), termites (Lewis et al. 1997, Brooks et al. 2003), and more recently fire ants (Lin et al. 2011) and bed bugs (Pfiester et al. 2008). Pfiester et al. (2008) found canine detection teams were 98% accurate at detecting as few as one bed bug and had no false indications using planted bugs in hotel rooms. Their study demonstrated the ability of bed bug scent dogs to detect low numbers of bed bugs with a high degree of accuracy under controlled conditions. The researchers also worked directly with a highly skilled canine scent detection trainer who provided both the bed bug detection dogs and conducted the inspections. As a result, the conclusions may not translate into real world inspections conducted in naturally infested dwellings..

The accuracy of canine scent detection for bed bugs is especially important for two obvious reasons. First, the high cost of canine detection services dictates that a higher detection rate should be provided compared to other available detection methods. Secondly, any false positive (indicating the presence of bed bugs when bed bugs are non-existent) can result in unnecessary application of pesticides and control costs along with disturbance of work and daily life. The objective of this study was to evaluate the performance of trained canines for detecting bed bugs under natural field conditions. We hypothesized that: 1) average detection rate is much lower than 95%; 2) average false positive rate is greater than 10%; and 3) detection and false positive rates vary significantly between inspections and teams.

Materials and Methods

Apartments

High-rise affordable housing communities located in Newark and Jersey City in New Jersey with current bed bug activity were selected for Experiments I-III. The apartments were either studio, one or two bedrooms and were occupied by elderly residents. Presence/absence of bed bugs in experiments with preselected apartments (Experiments I and II) was determined by placing an average of 28 Climbup® interceptors (Susan McKnight, Inc., Memphis, TN), hereafter referred to as interceptors, in each apartment for 14 d plus visual inspections of the apartment if no bed bugs were captured in the interceptors. Apartments were not preselected in Experiment III. For this experiment, monitoring with interceptors and visual inspection was performed post canine inspection. The residents were informed of the inspections and given a preparation list prior to the canine inspection. In a few apartments where exposed insecticide dusts were present, the researchers vacuumed out the dusts prior to canine inspections. After canine inspection, monitoring with interceptors and/or visual inspections were conducted in all units where the canine scent detection results differed from the expected results based upon detailed records of the current and previous infestation history of all apartments in the building. Low-rise garden style apartments (studio, one or two bedroom) located in New Jersey were used in Experiment IV. Apartment sizes in Experiment I ranged from 28-74 m^2, 48 m^2 for all apartments in Experiments II, and 59-74 m^2 for Experiment IV.

Canine Detection Teams

A total of 11 detection teams participated in three experiments. Teams selected were all within 322 km of the inspection sites. Five teams were from New York City, five from New Jersey and one from Maryland. Among them, two teams were selected based upon the recommendation of a highly respected canine scent detection trainer. Four teams were selected based upon their prominence in the bed bug detection industry. Another four teams were selected based upon an internet search and one team volunteered to participate in the study. All of the companies claimed or implied inspection accuracy of $\geq$ 95%. Prior to the start of the study, companies had been providing bed bug dog detection services for an average of 2.4 yr. The average length of time that dogs and handlers had been working together as a team was 1.1 yr. Additional background information for the teams is summarized in Table 2.1.

Table 2.1. Background information of the canine teams evaluated in this study.

Team	Original training facility	Background of handler prior to bed bug detection	Team working together (mo)	Certifi-cation	Type of reward	On or off lead	Dog breed	Sex of dog	Age of dog (mo)
1	Michigan	Pest Control	12	IAOCPI[1]	Food	On lead	Lab/ Collie mix	F	40
2	Michigan	Pest Control & termite detection dog/handler	24	IAOCPI	Food	On lead	Beagle/ Pug mix	M	36
3	Kansas	Hardware business	5	NESDCA[2]	Food	On lead	Terrier/ Pointer mix	M	30
4	Florida	Property Manager	14	None	Food	On lead	Golden Retriever	F	24
5	Alabama	Pest Control	24	WDDO[3]	Food	On lead	Coon-hound/ Pointer mix	M	42
6	Florida	Police Officer	36	WDDO	Food	On lead	Beagle/ Jack Russell mix	M	60
7	Florida	Pest Control	6	NESDCA	Food	On lead	Beagle	M	11
8	New Jersey	Pest Control	6	None	Food	On lead	Black Lab	F	30
9	Florida	Pest Control	20	NESDCA	Food	On lead	Beagle	M	52
10	North Carolina	Equestrian care	8	None	Play & Toy	Off lead	Black Lab	M	30
11	Company employed trainer	Military K9 bomb dog handler	2.5	None	Play	Off lead	Yellow Lab	M	12

[1] International Association of Canine Pest Inspectors.

[2] National Entomology Scent Detecting Canine Association.

[3] World Detector Dog Organization.

Determination of Accuracy of Dog Inspections

Accuracy of a dog/handler team was measured by two independent variables; 1) detection rate and 2) false positive rate. Each was equally important in determining the overall accuracy of a team's inspection. The higher the detection rate and lower the false positive rate during a given inspection, the more accurate the team was. The "detection rate" was the number of apartments with confirmed bed bug activity in which the dog alerts, divided by the number of apartments with confirmed live bed bug activity. The "false positive rate" was the number of apartments without confirmed bed bug activity in which the dog alerts, divided by the total number of apartments in which live bed bug activity could not be confirmed. Confirmation of bed bug activity was based upon 1) pre-inspection conducted by researchers within 2-4 d prior to the initial dog inspection, 2) post-inspection in apartments with bed bug counts of ≤ 5 bugs during pre-inspection to re-confirm the presence of bed bugs, and 3) post-inspection in apartments with alerts by dogs, in which bed bug activity was not previously known to exist.

Experiment I. Blind Evaluation in Preselected Apartments: Eight canine scent detection teams (Teams 1-8) belonging to seven companies were evaluated. The experiment was conducted in July 2011 in an apartment complex consisting of four separate buildings within two blocks of one another in a housing complex in Jersey City. Each firm was contacted by a representative of the housing authority to request a canine scent inspection of 24 apartments. The firms were unaware that they would be evaluated by a team of researchers. The seven firms quoted an average $757 ($480-1,000) for inspecting 24 units. A total of 48 apartments were selected for inclusion in the experiment and divided into two groups of 24, each group with a similar number of infested and non-infested apartments. The number of studio, one bedroom and two bedroom apartments inspected was similar in each group with 4, 19 and 1 in group 1 and 6, 19 and 2 in group 2. The infested apartments in each group were also similar in level of infestation (Table 2.2).

Table 2.2. Background information of the apartments inspected by canine teams in Experiment I.

Status of bed bug activity	Number of apartments Group 1	Group 2
Previously infested within last 2 years	5	5
No history of bed bug activity	7	9
Low level bed bug activity (< 10 bed bugs)*	8	7
Moderate level bed bug activity (11-50 bed bugs)	3	1
High level bed bug activity (51-73 bed bugs)	1	2
Total number of apartments	**24**	**24**

*Bed bug counts were based on interceptors placed for 14 d.

Inspections began within 3 d after the apartments were selected. Two days prior to the inspections, the apartments were prepared following the requirements listed in Table 2.3. The apartments were inspected over four consecutive days. The mean daily high outdoor temperature over the 4 d of inspections was 33.4 ^{o}C and ranged from 30.6 to 35.0 ^{o}C (http://www.ncdc.noaa.gov/). The temperature within each of the inspected apartments was not recorded. However, the apartment buildings were very hot inside because hallways were not air conditioned and few residents used air conditioning. For those residents that did use air conditioning, all teams required the air conditioning and fans to be turned off during the dog inspections (Table 2.3).

Table 2.3. Required preparations prior to dog inspections in Experiment I.

Team	Pesticides	Cleaning	Fans & air conditioning	Tobacco smoke	Pets	Items to remove
1 & 2	Na*	Vacuum under beds, furniture & floor	Off during inspection	Na	Dogs out, cats in crate or locked in bathroom, remove pet toys, food bowls & litter boxes	Na
3	Na	Na	Off 20 min prior to inspection	Na	Dogs out, Cats crate or locked in bathroom,	Potpourri, air deodorizers
4	Na	Na	Off during inspection	Na	Na	Na
5	Na	Vacuum apt. thoroughly	Off during inspection	Na	Na	Na
6	Na	Na	Off during inspection	Na	Na	Na
7	No essential oils within 30 d of inspection; no visible dust residues; provide list of any pesticides used in last 30 d	Na	Off during inspection	No smoking within 2 h of inspection	Dogs out, cats crate or locked in bathroom, remove pet toys, food bowls & litter boxes	Na
8	Na	Na	Off during inspection	Na	Dogs out, cats crate or locked in bathroom,	Na

*Na: no requirement.

Post-inspections were conducted in apartments in which bed bugs were not detected before the canine detection, but alerts were recorded during the canine inspection. Inspections were also conducted in apartments with pre-counts of ≤ 5 bugs to verify that bed bugs were still present. Post-inspections consisted of visual inspection of the entire apartment with emphasis in areas where alerts were recorded. If no bed bugs were found, interceptors were installed throughout the apartment for 14 d. Units where dogs alerted, but bed bugs were not found during both pre- and post-inspections were classified as false positives. Approximately 12 mo after the canine scent inspection, all apartments with false positives were inspected by placing interceptors under the legs of beds and upholstered furniture for 14 d, followed by a thorough visual inspection of beds and furniture if no bed bugs were observed in the interceptors.

Experiment II. Informed Inspection of Preselected Apartments: Subsequent to Experiment I, a similar experiment was conducted in August 2011 in Newark, New Jersey. This experiment was to obtain data on additional canine teams and to evaluate consistency in performance. Four scent detection firms were evaluated. Two of them (Team 1 and 4) were from the first study with the intention of examining their consistency. They were selected based on their high detection rate or low false positive rate. Two new firms (Team 9 and 10) were selected based upon their strong reputation within the bed bug scent detection industry.

The apartment complex consisted of two five-story buildings (A and B) separated by a 60 m corridor. Twenty apartments were selected from the buildings using a combination of historical pest control records and pre-monitoring as in Experiment I. Among them, a mean bed bug count of 33 (range: 2–122) was obtained based upon interceptor trap catch in 11 of the 12 infested apartments. Inspections were conducted over 2 d. The maximum temperatures during the two days of inspections were 35.6 and 33.3 ^{o}C, respectively. The inspection time spent in each apartment, the number and location of alerts in each apartment were recorded by the researchers. Within 24 h after the last canine scent team's inspection, post-inspections were conducted in a similar manner as in Experiment I. The only difference was that pre-counts in Experiment I were based on a 14-d monitoring interval with interceptors, while in this experiment interceptors were inspected after 7 d. If no bed bugs were found, then the interceptors were inspected again at 14 d and a visual inspection was followed if no bugs were found in interceptors. Approximately 12 mo after the canine scent inspection, all apartments with false positives were inspected again by placing interceptors under the legs of beds and upholstered furniture for 14 d, followed by a thorough visual inspection of beds and furniture if no activity was observed in interceptors.

Experiment III. Informed Building-wide Inspection: The purpose of this experiment was to examine the performance of two canine teams when conducting a large scale building-wide inspection. The previous two experiments included small number of units and high proportions of infested units which may not reflect real world situations that are typically encountered by canine scent firms. In addition, the apartments inspected in Experiments I and II were spread out over five or more floors and two or more buildings. In this experiment, detection teams inspected a large number of apartments in a continuous block. This allowed the detection team to move from one apartment to the next in a continuous fashion, eliminating the disjointed nature of the previous two experiments. The experiment was conducted in the same apartment complex used in Experiment II; however, apartments were not pre-monitored. Team 9 from Experiment II inspected 102 apartments in Building A and a new team (Team 11) inspected 106 apartments in Building B.

The inspections were conducted during September and October 2011. Unlike all other teams, the dog handler of team 11 used visual inspection after each dog alert in an effort to confirm the alert and therefore spent much longer time than team 9. The maximum outdoor temperature on the day building A was inspected was 23.3 ^{o}C. The maximum daily high temperature outdoors over the three days of inspections for building B was between 19.4 and 30.0 ^{o}C. Post-inspections were conducted in: 1) apartments with an alert, and 2) apartments without alerts but had previous history of bed bug activity within the past 24 mo, based upon historical records. These inspections were carried out in a similar manner to those in the Experiment II.

Experiment IV. Detecting Planted Hides in Apartments: To determine if a higher detection performance could be achieved under controlled conditions, team 10 from Experiments I and II was selected for this experiment. This team was selected because they had a very low detection rate (15%) in Experiment II and was willing to participate in this experiment. The experiment was conducted in four one or two bedroom apartments. No previous bed bug activity had ever been reported in the buildings where the apartments were located. Three of the apartments were fully furnished. Two of these three apartments were occupied and one was a model apartment used for showing to potential renters. The fourth apartment was recently vacated with some furnishings left behind including two mattresses, two box springs, a china closet and 6 dining room chairs. Bed bug hides in fabric sachets were prepared in the laboratory the day before the experiment. Nitrile gloves were worn while handling the materials used to make the hides. The fabric sachets were approximately 6.5 cm^2 and were made by folding a fine mesh nylon fabric (Party Time White Chiffon Fabric, Walmart, Princeton, NJ) in half and sealing the sides with hot glue to create a one square inch envelope open on one end. A paper harborage was inserted inside each sachet. For control hides, the end of the sachet was then sealed with hot glue. For live hides, five live adult male bed bugs were placed in each sachet before the sachet was sealed (Fig. 2.1).

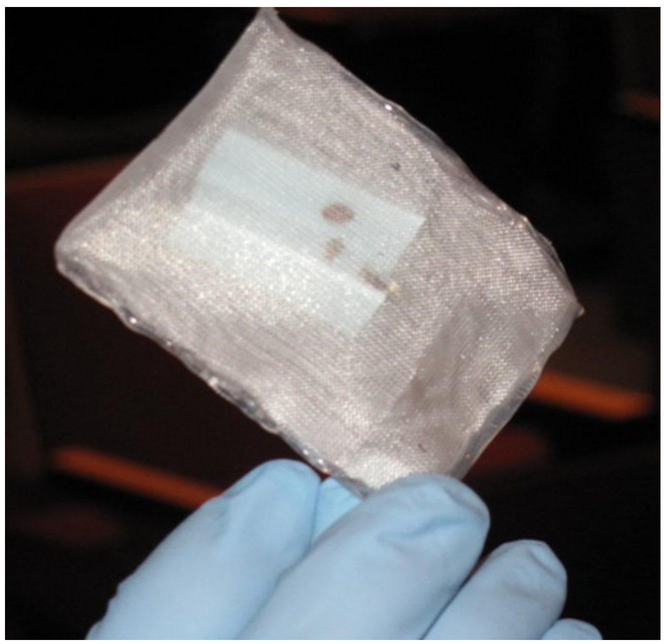


Figure 2.1. A sealed sachet with five adult male bed bugs used for evaluating bed bug scent dogs in Experiment IV.

All control hides were stored in a plastic container and sealed before live bugs were handled. The live hides were stored in a separate plastic container. All sachets were stored overnight in the laboratory at room temperature and then transported to the study site. Each apartment received three control and three live hides. The hides were placed approximately 1 h before the inspections. Latex gloves were worn to handle and place hides. One researcher placed the control hides and another researcher placed the live hides to avoid any cross contamination. Live hides were examined to ensure that all of the bugs were still alive at the time of placement in apartments. The hides were placed in a variety of areas including the upholstered furniture, beds, night tables, dressers, and closets. The control hides and live hides were placed in separate rooms to control for confusion over positive and false positive alerts. The exact locations of all alerts were recorded. The scent dogs tested had no prior exposure to placement of hides by either researcher prior to this experiment.

In all four experiments each team was instructed to inspect the entire apartment including the living room, bedroom, hallways, closets, bathroom and kitchen. Teams were reminded of this again during their inspection if it was observed that their search pattern did not include all areas within the apartment.

Statistical Analysis

The bed bug count data were ranked and then the association between the rank and the team's detection result (yes or no) was analyzed. Four teams (team 1, 4, 9 and 11) which performed inspections in 21-25 infested apartments were examined by Wilcoxon rank sum test (PROC NPAR1WAY in SAS software, SAS Institute 2011). The relationship between a team's detection rate and the false positive rate was analyzed by regression analysis (PROC REG in SAS software). The relationship between detection rate and a team's experience (number of years the dog and handler worked together) and certification status (yes or no) was also analyzed by regression analysis. For the four teams that performed multiple inspections (different days or locations) results were combined for regression analysis.

Results

Experiment I. Blind Evaluation in Preselected Apartments

Results for Experiment I are summarized in Table 2.4. The mean (min, max) time for detection teams to inspect an apartment was 3.2 (1.2, 6.0) min. One team's dog was too tired from the heat to inspect the last apartment. Another team was unable to inspect an apartment because the resident died the night before the inspection. Neither of these units had bed bugs. Bed bugs were detected in four units that were not known to have bed bugs prior to the canine inspection, however each was detected by only one of the 3 or 4 dogs that inspected each of these apartments. The eight teams had mean (min, max) detection rate of 47 (10, 88)% and false positive rate of 19 (0, 50)%.

Table 2.4. . Canine inspection results in Experiment I.

Team	Group	# apts. inspected	Avg. time per unit (min)	Time on break (min)	# of infestations*	# of infestations detected	# of apts. with false positives	Detection rate (%)	False positive rate (%)
1	1	14	3.5	5	8	7	1	88	17
2	1	10	5.4	3	4	3	3	75	50
3	1	24	2.7	24	12	6	2	50	17
4	1	24	4.0	0	12	6	0	50	0
5	1	24	2.5	0	10	5	4	50	29
6	2	23	2.7	0	10	3	2	30	15
7	2	23	6.0	41	12	3	2	25	18
8	2	24	1.2	7	10	1	1	10	7

*The number of infestations is based upon a combination of interceptor trap catch and visual inspection.

Only 4 out of the 22 infestations were detected by all of the teams that inspected them. Bed bug counts from traps and visual inspection in the 4 apartments detected by all firms were 25, 31, 68 and 73. Another four apartments with 1, 25, 38 and 62 bed bug counts were missed by all teams that examined those units, indicating the level of infestation is not a predictable factor of probability of being detected by canines. Residents in two infested apartments owned pets: one owned a dog, another owned a cat. The apartments with the dog and cat had bed bug counts of 38 and 62, respectively, but no alerts were recorded by any of the three teams that inspected these two apartments. Among the apartments with (n=10) or without (n=14) previous bed bug history, false positive alerts occurred in 50 and 56% of them respectively, indicating false positive alerts are not positively correlated with previous bed bug infestation history. Over the course of the next 12 mo no bed bug activity was reported or detected in any of the apartments where false positives were recorded.

Experiment II. Informed Inspection of Preselected Apartments

Results for Experiment II are summarized in Table 2.5. The mean (min, max) time for detection teams to inspect an apartment was 5.3 (1.9, 8.0) min. One of the four canine teams (Team 1) identified an additional infestation not known to have bed bugs prior to the inspection. A single bed bug was detected based upon interceptor trap catch during post-inspection and monitoring of the apartment, bringing the total number of apartments with bed bug activity to 13. The four teams had an average detection rate of 50 (15, 77)% and false positive rate of 32 (14, 57)%.

Table 2.5. Canine inspection results in Experiment II.

Team	Day and time of inspection	# apts. inspected	Avg. time per unit (min)	Time on break (min)	# of infestations*	# of infestations detected	# of false positives	Detection rate (%)	False positive rate (%)
1	Day 1 PM	20	5.0	35	13	10	4	77	57
4	Day 2 AM	20	5.0	40	13	9	3	69	43
9	Day 2 PM	20	1.9	0	13	5	1	38	14
10	Day 1 AM	20	8.0	20	13	2	1	15	14

*The number of infestations is based upon a combination of interceptor trap catch and visual inspection.

Only one infested apartment with a pre-count of 68 bed bugs was detected by four teams. Two infested apartments with pre-counts of 6 and 20 bugs were missed by all four teams. Overall, 57% (4 of 7) of the apartments without bed bugs were alerted in by one or more teams. During post-inspections, visual evidence of previous bed bug activity was observed in all but one of the apartments where false positive alerts were recorded. The team with highest detection rate (77%) also had the highest false positive rate (57%). Conversely, the team with lowest detection rate (15%) also had the lowest false positive rate (14%). Over the course of the next 12 mo no bed bug activity was reported or detected in any of the apartments where false positives were recorded.

Experiment III. Informed Building-wide Inspection

The results of Experiment III are summarized in Table 2.6. Team 9 completed the inspection of 102 units in a single day. The mean (min, max) working time to inspect each apartment, excluding all down time was 1.2 (0.5-2.0) min. The team took one 45 min break during the inspection. The mean time required for inspecting each apartment including the break and time between units was 2.7 min. The team only detected two of nine infested apartments, which had 2 and 14 bed bugs, respectively. The mean (min, max) number of bugs in the seven missed apartments was 12 (1, 52). Thirty-four of the 93 non-infested apartments had previous infestation history. Seventy-one percent (5 out of 7) of the false positives were in apartments with prior history of bed bug activity. Approximately 3 mo after the canine inspection, four bed bugs were detected in one of the seven units previously recorded as a false positive, thus the possibility that bed bug activity was present at the time of the dog inspection cannot be ruled out, making the ranking of this apartment as a false positive questionable.

Table 2.6. Canine inspection results in Experiment III.

Team	# days to complete inspection	# apts. inspected	Mean time (min) to inspect per apt.*	# of infestations+	# of infestations detected	# of false positives	Detection rate (%)	False positive rate (%)
9	1	102	2.7	9	2	7	22	8
11	3	106	10.6	23	10	4	43	5

*Including down time (breaks and travel between apartments).

+The number of infestations is based upon a combination of interceptor trap catch and visual inspection.

Team 11 completed the inspection of 106 units in 3 d. The mean (min, max) working time to inspect each apartment, excluding all down time was 4.0 (1.0, 9.5) min. The team required 11 breaks totaling 7 h, to finish the inspections. The mean time required for inspecting each apartment including the break and time between units was 10.6 min. The team detected 8 of 21 known infestations and detected bed bugs in another two apartments where bed bugs were not known to exist prior to the team's inspection, bringing the total number of apartments with bed bug activity to 23 and the number of these apartments detected by the team to 10. The mean (min, max) bed bug count in the 10 apartments detected was 5.0 (1, 15).

The mean (min, max) bed bug count in the 13 missed apartments was 6.1 (1, 18). Seventeen of the 83 non-infested apartments had previous infestation history. Of the four false positive alerts, two occurred in apartments with previous activity. Over the course of the next 12 mo no bed bug activity was reported or detected in any of the apartments where false positives were recorded.

From Experiments I-III, there were a total of 16 inspections (Team 11 was considered having performed three inspections). To calculate the overall accuracy of the inspections we omitted two of the inspections (Teams 2 and 2nd day inspection by team 11) because the total number of infested units was only 4 in each. The mean detection rate and false positive rate for the 14 remaining inspections were 44 (10-100)% and 15 (0-57)%, respectively. False positive alerts occurred nearly equally in apartments with bed bug history (49%) as in units with no infestation history (51%). Of the 67 apartments with bed bug activity, 93% (62 out of 67) were detected by placing interceptors for 7 or 14 d.

We analyzed the relationship between the detection rate and false positive rate. Teams 1, 4, 9 and 11were evaluated on multiple days. The combined detection rate and false positive rate were used for these teams. Team 2 was excluded due to its small number of inspections. A team's detection rate was positively correlated to its false positive rate ($F = 7.6$; $df = 1, 8$; $P = 0.02$; $R^2 = 0.49$) (Fig. 2.2). There was no significant relationship between the detection rate and the length of time the team had been working together ($F = 0.36$; $df = 1, 9$; $P = 0.56$) and whether the team was certified ($F = 1.4$; $df = 1, 9$; $P = 0.26$) (Table 2.7).

Table 2.7. Relationship between team profile and performance of the inspections.

Team	# years in bed bug scent detection business	Team working together (mo)	Certification	Detection rate (%)	False positive rate (%)
1*	2	12	IAOCPI[1]	81	38
2	2	24	IAOCPI	75	50
3	1.5	5	NESDCA[2]	50	17
5	3	24	WDDO[3]	50	29
11*	0.8	2.5	None	43	5
4*	1.2	14	None	60	16
9*	4	20	NESDCA	32	8
6	3	36	WDDO	30	15
7	3	6	NESDCA	25	18
8	5	6	None	10	7
10	0.75	8	None	15	14

*Total detection and false positive rates from multiple inspections.

[1] International Association of Canine Pest Inspectors.

[2] National Entomology Scent Detecting Canine Association.

[3] World Detector Dog Organization.

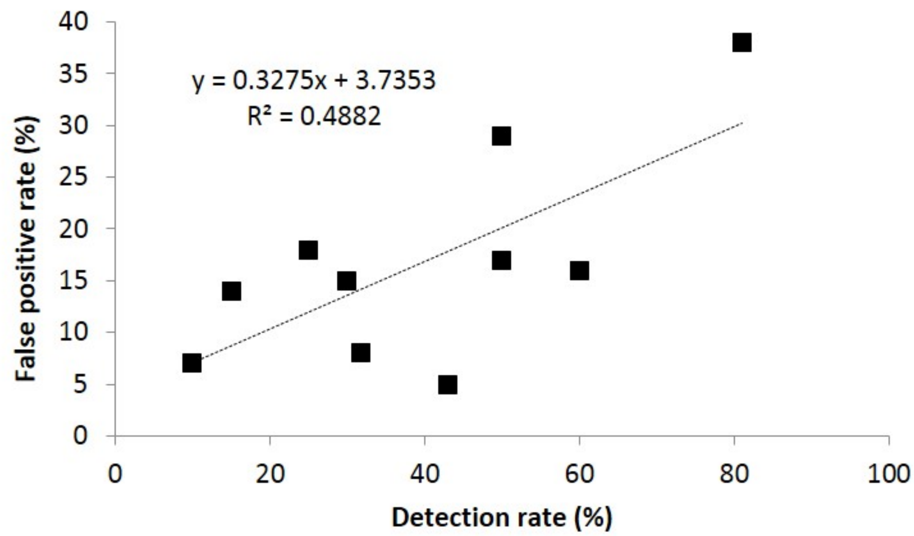


Figure 2.2. Relationship between a dog team's detection rate and false positive rate based upon ten teams.

Teams 1, 4, 9 and 11 inspected more than 15 infested apartments and were used to analyze the relationship between bed bug population level and the probability of being detected by dogs. For each of these four teams, there was no significant relationship between the detection result (yes or no) and the rank of the bed bug count (team 1: χ^2 = 12.8, df = 14, P = 0.80; team 4: χ^2 = 16.3, df = 17, P = 0.68; team 9: χ^2 = 17.8, df = 16, P = 0.29; team 11: χ^2 = 12.5, df = 12, P = 0.45). Teams 1, 4 and 9 were evaluated twice. The accuracy of these teams varied greatly between inspections (Table 2.8). Team 11 spent 3 d to finish inspecting 106 apartments in Experiment III, inspecting 31, 39, and 36 apartments, respectively. We considered these inspections separate events for the purpose of evaluating consistency of inspections conducted by the same team on different days. The detection rate changed greatly during the three inspections (Table 2.8).

Table 2.8. Consistency of canine detections in detecting bed bug infestations.

	# of apartments inspected / # of infested apartments			**Detection rate/ False positive rate (%)**		
Team	Insp. # 1	Insp. # 2	Insp. # 3	Insp. # 1	Insp. # 2	Insp. # 3
1	14/8	20/13	-	88/17	77/57	-
4	24/12	20/13	-	50/0	69/43	-
9	20/13	102/9	-	38/14	22/8	-
11	31/7	39/4	36/12	100/0	25/11	17/0

Experiment IV. Detecting Planted Hides in Apartments

Team 10 detected 2 of 3 live hides and did not alert on any of the controls in the first apartment. In the 2nd apartment, all three of the live hides were detected with no false positives. In the 3rd apartment, two of three live hides were detected and one control was falsely alerted upon. In the 4th apartment, detection rate and false positive rates were both 100%. Overall the team detected 83% (10 of 12) live hides and falsely alerted on 25% (4 of 12) of the control hides. The same team in Experiment II had 15% detection rate and 14% false positive rate.

Discussion

This study is the first evaluating the accuracy of commercially available bed bug canine detection teams under field conditions. We found the detection rates and false positive rates varied greatly among canine detection teams and within teams evaluated on different days. A team's detection rate is positively correlated with its false positive rate. There was no significant relationship between bed bug infestation level, the team's experience, or certification status of teams and the detection rates. A detection rate of ≥ 90% and a false positive rate of ≤ 10% occurred in only 1 out of 16 inspections (Team 11) and this team spent a much longer time than the other teams to inspect each apartment. It should also be noted that this team's perfect performance on the 1st day is negated by its disappointing low level performance the following two days. Overall, the team only detected 43% of the infestations in the building during its three days of inspection. The accuracy of the 11 canine detection teams evaluated was much lower than that reported in controlled environments (Pfiester et al. 2008). The mean detection and false positive rate in our study was 44 and 15%, respectively, compared to 98 and 0%

using controlled hides conducted in hotel rooms. Consistent with this, we observed a marked difference between performance of a team evaluated in naturally infested apartments and in apartments with controlled hides.

Brooks et al. (2003) suggested, for detection of termites, that it is "not unreasonable to expect a properly trained dog to meet a minimum standard with a positive indication rate of ≥ 90% and a false positive rate of ≤ 10%". The only other study investigating the accuracy of trained dogs for termites reported a mean detection rate of 81% and false positive rate of 28% in a laboratory setting (Lewis et al. 1997). Our observed mean detection rate of 44% and false positive rate of 15% is more in line with that reported by Lewis et al. (1997). Furthermore, mean detection and false positive rates in our study were similar regardless of whether or not detection firms were aware they were being observed. When judged based on 90% detection rate, only one out of 16 inspections meet the proposed standard by Brookes et al. (2003). When judged by 10% false positive rate, only 5 out of 16 inspections meet the expected standard by Brookes et al. (2003). When both standards are used to judge the canine teams' performance, all but one of the 16 inspections fall short of expectations. Comparing to the 93% detection rate from installing interception devices for 7-14 d, the results of the canine inspections were much less effective at detecting infestations. Moreover, use of monitoring devices eliminates false positives, unless the inspector is not properly trained to distinguish bed bugs from other arthropods.

We attempted using an alternate method to measure a team's effectiveness: the "total correctness" (TC). TC is the total of correct positive alerts plus correct "non-alerts" divided by the total number of units inspected. Using TC can be very misleading. This is illustrated by team 9 who inspected 102 apartments in Experiment III. The team only detected 2 of 9 infestations (22% detection rate) and falsely alerted in 7 apartments. The TC was 86% however, the very low detection rate and seven falsely identified infestations are diluted due to the large sample size of the apartments inspected (n=102). We do not believe TC should be used as a measure of accuracy due to its misleading nature. Instead, we believe it is necessary to consider both detection rate and false positive rate to evaluate a team's effectiveness.

The question remains, why the scent detection teams that we evaluated performed so poorly? An obvious difference between performance of a canine team under controlled and field conditions is that errors by dogs and handlers are identifiable and correctable in a controlled setting, while the natural field setting is very complex with odors from many different sources where errors can easily occur, go unidentified and thus remain uncorrected, reinforcing the incorrect behavior. This creates challenges in the ongoing training and evaluation of a team's performance. In a study with wild brown tree snakes, Savidge et al. (2011) suggested dogs were using scent cues from containers and/or humans that placed hides to help detect the target scent. In order to overcome this problem, they fed snakes dead mice with radio transmitters inside and allowed the snake to hide on their own. In our controlled hide study, the dog exhibited a learning behavior over the course of four inspections in apartments with controlled hides. The dog was able to pinpoint the exact location of the live hides and recorded no false positives in the first two apartments. By the 3rd apartment the dog alerted on one control hide and by the 4th apartment, it alerted to all of the live hides and all control hides. There were no false positives in areas where there were no hides. By the 4th apartment, it appears the dog may have changed the target scent profile from live bugs, to that of the sachet, the latex gloves worn when placing out the hides, or both. Subtle, but significant changes such as this can be identified and corrected under controlled conditions. However the same is not always true under field conditions. All of the firms evaluated had dedicated ongoing maintenance training varying in degree of complexity, however none used field sites with naturally existing infestations to train their teams. Although, only one team was evaluated under both controlled and field conditions, when asked about their in-house training programs, all of the teams evaluated indicated that they have > 95% accuracy in their in-house maintenance training exercises, which was not reflected in the field results observed in our study. We suggest that self-evaluating the dogs in naturally infested apartment complexes, offices, hotels, etc. could help improve the accuracy of the inspection teams.

Other factors such as handler bias and unintentional handler cues (Gazit et al. 2005), confusing combinations of scents (Waggoner et al. 1998, Lit and Crawford 2006), insufficient training for all situations (Gazit et al. 2005, Lit and Crawford 2006, Lit et al. 2011), environmental conditions (Smith et al. 2003), level of maintenance training (Cablik and Heaton 2006) and enhanced distractions inherent in applied settings (Lit and Crawford 2006) can influence the team's performance. Smith et al. (2003) suggested the heat may have affected the accuracy of dogs to detect San Joaquin kit foxes in their study. In order to cool the body, dogs pant, however while panting they are unable to sniff. Gazit et al. (2003) demonstrated an inverse relationship between increased panting and the efficiency of dogs to detect explosives. In our study, Experiments I and II were conducted when the average daily high temperature was 33.4-34.5 ^{o}C. The hallways and most apartments were not air conditioned, creating hot conditions. During these inspections some of the dogs showed fatigue and increased panting, while others showed little to no visible affect from the hot weather. In Experiment I, the dog from team 3 became so fatigued that it was only able to inspect 23 of the 24 apartments. The team performed poorly with a 25% detection rate and 18% false positive rate. The handler for team 1 stopped the inspection after the 5^{th} apartment so he could remove his dog from the building to rest in his air conditioned vehicle. Interestingly, of the eight teams evaluated in Experiment I, team 1 had the highest detection rate (88%) with a false positive rate of 17% despite the hot conditions. Gazit et al. (2003) and Garner et al. (2001) showed that dogs can be trained to adjust to working under severe physiological conditions. Companies offering canine scent detection for bed bugs must understand the limitations of their dogs and incorporate appropriate conditioning exercises for the various types of environments and conditions they are likely to encounter. Alternatively, they should refuse inspections when environmental conditions are not conducive for a quality inspection.

A context shift effect (Gazit et al. 2005) can also occur between maintenance training and real world field inspections. For example, if routine training exercises never exceed 30 min during which time the dog is accustomed to being rewarded at least once, the dog may exhibit decreased attention once 30 min have elapsed without reward during a field inspection (Oxley and Waggoner 2009). A context shift, such as this, could be particularly problematic during a large scale inspection where only a few infestations exist. Conversely, a context shift effect could also occur when inspecting a facility with a much higher infestation rate than that in training exercises. Experiments I and II were done with high infestation rates (42-65%). After learning the results of their inspections, 4 of 10 handlers expressed concerns that the high infestation rate was greater than what they normally use in training exercises and may have negatively affected their results. While infestation rates should have no bearing on the accuracy of an inspection, from a context shift perspective, confusion can result when target scent is present at much greater frequencies than what the dog and/or handler are accustomed to. Based upon our observations during inspections in our study it was not uncommon for handlers to begin second guessing the dog after it had alerted in what the handler believed to be too many units, creating handler bias which undermines the inspection. Thus, maintenance training should not only be done within the context of the environment that is to be inspected but should vary in duration from short to very long, and include scenarios in which target scent is 1) not present (no reward), 2) present at a typical frequency, and 3) present at high frequencies.

Lit et al. (2011) illustrated that preconceived beliefs of handlers can influence the outcome of an inspection, leading to inaccurate results. False positive alerts occurred in some of the apartments where old evidence of fecal spotting, carcasses and exuvia were readily visible and recognized by handlers during the inspection. This may have led to unintentional cues to the dog by the handler. Some of the handlers we worked with also demonstrated a preconceived belief regarding where bed bugs were likely, or not likely, to be found. In our study, all teams paid close attention to bedrooms and living rooms but 7 of the 11 teams did not plan on including kitchens, bathrooms and all closets in their inspection, indicating to us that bed bugs were not likely to be found in these areas. Five of the 7 teams included these areas in their inspection after we requested them to do so; still they paid less attention to areas away from beds and upholstered furniture. Two (teams 3 and 5) of the 7 teams ignored our requests to inspect all rooms, halls and closets because they were confident bed bugs would not be found in these areas. It is possible that some of the missed detections were the result of biased search patterns in which areas where the handler did not believe bed bugs were likely to be present were ignored. In 20 of 67 apartments bed bugs were not detected in interceptors or through visual inspection at beds or upholstered furniture during pre- and post-inspections but instead were only captured in interceptors located in less predictable locations such as kitchens, bathrooms, hallways and hall closets. During the blind study (Experiment I), after requesting one handler to inspect the entire apartment thoroughly, the handler informed us that if bugs are present they will be in bedroom or living room. This team missed all the three units where bed bugs were only observed or captured in interceptors located in areas outside of the bedroom and living room (kitchen, bathroom, hall, or closets) but detected 67% (6 of 9) of the apartments where bed bug activity was observed in the bedroom and/or living room.

The term “team” is used because the accuracy of the inspection is dependent upon the ability of the dog to detect the target scent and the handler’s ability to manage the inspection and interpret or “read” the dog’s behavior. The alertness of the team, responsiveness of the dog to the handler, and the handler’s ability to interpret the dog’s behavior can affect the inspection (Furton et al. 2010). This was illustrated during the three day inspection by Team 11. The first day inspection by this team was perfect, with the dog detecting all 7 apartments with activity and no false alerts, illustrating the ability of a team to operate with a high degree of accuracy under natural field conditions. On the second day, the dog alerted in the exact location where bed bugs were present in all four of the apartments with bed bug activity, however three of the four alerts were dismissed by the handler who interpreted that the dog was "playing" him and was just looking for a reward. Thus on the second day, the dog continued to work with a high degree of accuracy, however the handler did not. By the third day the dog alerted in only 17% (2 out of 12) apartments with bed bug activity, a marked difference from the previous two days. Based upon our observations, both the dog and the handler seemed disinterested during these inspections. Gazit et al. (2005) suggested that disinterest on the part of the handler could be unwittingly transmitted to the dog resulting in a decreased motivation by the dog to search.

False positive alerts can result in significant direct and indirect costs. A total of 28 false positives were recorded by one or more teams in this study. Over the course of the next 12 mo the presence of bed bugs was confirmed in only one apartment. Had all of these units been treated based upon the results of the dog inspection the direct treatment costs are likely to have exceeded $13,000 based upon the typical treatment cost of $463-$482 per apartment reported by Wang et al. (2009). In addition to the direct financial impact, other potential costs include unnecessary exposure to pesticides, property loss from items discarded and damage to reputation. Based upon the high false positive rates observed among the teams studied, confirmation of existing bed bug activity in areas of alerts seems reasonable and appropriate. Duggan et al. (2011) suggested that following an alert by detection dogs for cryptic species, employing a second step to confirm the presence of the target can increase the effectiveness and decrease costs associated with large scale inspections. Alerts which cannot be confirmed should not be considered positive for bed bug activity. Instead, they should be considered suspect and worthy of additional inspection/monitoring to determine if bed bugs are in fact present.

There is also a great disparity in the degree of formal training received between handlers of bed bug scent dogs compared to that received by handlers in law enforcement and the military who go through extensive training under the instruction of a qualified instructor. A minimum of 40 h of classroom training and 200 h of practical application are recommended for these military and law enforcement canine handlers (Furton et al. 2010). Only two of the handlers in our study had previous experience handling scent dogs, the rest received fewer than 40 h of combined classroom and hands on training with scent dogs, conducted at the training facility where their dog was purchased. Due to the small number of handlers with previous experience and/or extensive training we were unable to analyze the relationship between the degree of training and the quality of an inspection. This is an area where further research is required.

Furton and Myers (2001) suggested despite the fact that dogs are the most efficient, reliable and cost effective real time method for explosive device detection, operational complexities of dog handler teams coupled with limited scientific information on how the dogs function, as well as handler and dog training and operational deployment, makes the implementation of highly reliable and efficient detection teams less straightforward than analytical equipment. The low accuracy of trained dogs for bed bug detection suggests that the capability of dogs to determine presence/absence of bed bugs in natural conditions may be more limited than under controlled conditions. However, canine scent detection offers the only practical method for large scale inspections in nonresidential settings such as schools, office buildings, retail stores, theaters or mass transit, where thousands of square meters may require inspection and where bed bugs are less predictable making them more difficult to detect by other methods currently available. Thus there is an urgent need to develop better training and maintenance methods to improve detection rates and reduce false positives.

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CHAPTER THREE

Mark-Release-Recapture Reveals Extensive Movement of Bed Bugs (*Cimex lectularius* L.) Within and Between Apartments

Abstract

Understanding movement and dispersal of the common bed bug (*Cimex lectularius* L.) under field conditions is important in the control of infestations and for managing the spread of bed bugs to new locations. We investigated bed bug movement within and between apartments using mark-release-recapture (m-r-r) technique combined with apartment-wide monitoring using pitfall-style interceptors. Bed bugs were collected, marked, and released in six apartments. The distribution of marked and unmarked bed bugs in these apartments and their 24 neighboring units were monitored over 32 days. Extensive movement of marked bed bugs within and between apartments occurred regardless of the number of bed bugs released or presence/absence of a host. Comparison of marked and unmarked bed bug distributions confirms that the extensive bed bug activity observed was not an artifact of the m-r-r technique used. Marked bed bugs were recovered in apartments neighboring five of six m-r-r apartments. Their dispersal rates at 14 or 15 d were 0 – 5.0%. The estimated number of bed bugs per apartment in the six m-r-r apartments was 2,433-14,291 at 4-7 d after release. Longevity of bed bugs in the absence of a host was recorded in a vacant apartment. Marked large nymphs (3^{rd} – 5^{th} instar), adult females, and adult males continued to be recovered up to 57, 113, and 134 d after host absence, respectively. Among the naturally existing unmarked bed bugs, unfed small nymphs (1^{st} – 2^{nd} instar) were recovered up to 134 d; large nymphs and adults were still found at 155 d when the study ended. Our findings provide important insight into the

behavioral ecology of bed bugs in infested apartments and have significant implications in regards to eradication programs and managing the spread of bed bugs within multi-occupancy dwellings.

Introduction

The behavioral ecology of the bed bug (*Cimex lectularius* L.) in naturally infested dwellings is poorly understood. Much of what we do know dates back to research conducted nearly 50 years ago or more (Kemper 1930, Rivnay 1932, Johnson 1937, Mellanby 1939, Omori 1941, Usinger 1966). While there has been an increase in research as a result of the recent resurgence of bed bugs, research on their activity in naturally infested dwellings has been limited (Reinhardt and Siva-Jothy 2007).

Wang et al. (2009) used both visual inspection and an experimental pitfall-style trap placed under the legs of beds and furniture to quantify changes in the number of bed bugs in 16 infested apartments. Ninety-nine percent of the bed bugs found during visual inspection were associated with beds and upholstered furniture, however the pitfall trap catch suggested that a higher percentage of bed bugs were coming from areas off the bed than from on the bed. More recently, pitfall-style interceptor traps were shown to be effective at trapping bed bugs when placed in locations away from the beds and upholstered furniture (Wang et al. 2010, Wang and Cooper 2012, Potter et al. 2013b, Cooper et al. 2014, 2015a). These studies suggest that bed bugs can travel extensively within infested apartments, and that passive pitfall-style traps can be used to monitor bed bug movement both toward and away from host sleeping and resting areas.

Haynes et al. (2008) reported that under laboratory conditions bed bugs can travel up to 4.9 m in five minutes, and suggested that they can travel greater distances during the night, when they are active for hours at a time. Pfiester et al. (2009b) investigated aggregation behavior under laboratory conditions and suggested that first instar nymphs were the least likely developmental stage to disperse, and that recently fed adult females were the most likely to move away from aggregations. In another laboratory study, How and Lee (2010) examined distance traveled by various stages of the tropical bed bug (*Cimex hemipterus* (F.)), a species closely related to the common bed bug. They concluded that distance traveled varied significantly based upon the developmental stage, adult sex, and feeding status. Similar to Pfiester et al. (2009b), How and Lee (2010) concluded that early instars were the least likely to disperse and recently fed females were the most likely to disperse.

A limited number of studies have examined bed bug dispersal behavior under field conditions (Johnson 1937, Naylor 2012, Lehnert 2013). Naylor (2012)] provided evidence that both nymphs and adults of both sexes disperse based upon bed bugs captured on sticky traps located in a common hallway of an apartment building outside of two infested apartments. Lehnert (2013) found 18 lone bed bugs away from aggregations in eight infested apartments but did not report the distance between the nearest aggregation and the location of the lone bugs. Potter et al. (2013b) provided some insight into the bed bug movement within apartments by marking bed bugs with different colored paints, and then using pitfall traps and visual inspection to detect bed bug movement in two heavily infested residences. During the one week study, they found bed bugs that had moved up to 9.1 m away from their original resting location.

Active dispersal of bed bugs from infested apartments to neighboring apartments has been implicated as one of the causes for the spread of bed bugs within communities.

The spread of bed bugs from one unit to 68 units was tracked in a medical school housing facility over a 25 mo period (Doggett and Russell 2008). Similarly, bed bugs spread from a single apartment to 53% of the apartments in a 223 unit building in just 41 mo (Wang et al. 2010). In both studies, over 50% of the infested living units shared a common wall, floor or ceiling with another infested apartment (Wang et al. 2010, Doggett and Russell 2008). The first evidence that active dispersal may be in part responsible for widespread infestations was provided by Wang et al. (2010) who captured bed bugs in interceptor traps located behind the entry door inside infested apartments, as well as in traps located in the hallway just outside of infested apartments. Naylor (2012) also believed that bed bugs captured on sticky traps located in a common hallway of an apartment building had dispersed from a heavily infested apartment. Molecular studies have provided additional evidence in support of active dispersal of bed bugs between apartments (Booth et al. 2012, Saenz et al. 2013). Collection of bed bugs from infestations in different apartments within the same apartment building expressed low genetic diversity, suggesting that widespread infestations within apartment buildings were most likely to have resulted from a single introduction (Saenz et al. 2013). In another study using microsatellite DNA markers to screen bed bug populations in apartment buildings with widespread infestations, it was suggested that bed bugs were actively dispersing between neighboring apartments above, below, adjacent, or within a short distance of an infested unit (Booth et al. 2012). In spite of the existing evidence for active dispersal, absolute proof is still lacking.

In this study, we first evaluated the ability of interceptors in catching different bed bug developmental stages and the effect of marking procedure on mortality of bed bugs.

We then used m-r-r technique in both vacant and occupied apartments to study the movement of bed bugs within and between apartments, to estimate population size, and to examine the longevity of bed bugs in the absence of a host.

Materials and Methods

Ethics Statement

The field study received Rutgers University IRB approval (protocol # E11-766). Permission to perform the m-r-r study was granted from the housing authorities that participated in the study. Consent was obtained from residents whose apartments were used in the study and they were compensated either US $50 or $200 in appreciation for their time and cooperation.

Laboratory Bioassays

Reliability of interceptors for estimating bed bug population structure.

Climbup interceptors (Susan McKnight, Inc., Memphis, TN, USA) referred to hereafter as interceptors, were used throughout this study to sample bed bugs in apartments. We carried out two laboratory assays to examine: 1) if different stages and sexes are equally trapped by interceptors, and 2) determine the escape capability of bed bugs that had fallen into interceptors.

A strain of bed bugs collected between 2008 and 2010 from apartments in Indianapolis, IN, was used in the test. They were maintained in round plastic containers (4.7 × 5 cm) (Consolidated Plastics, Stow, OH, USA) with folded construction paper (Universal Stationers Supply Co., Deerfield, IL, USA) as harborages at 26 ± 1°C, 40 ± 10% RH, and a 12:12 h (L:D) photoperiod. They were fed weekly on defibrinated rabbit blood (Hemostat Laboratories, Dixon, CA, USA) using a Hemotek membrane-feeding system (Discovery Workshops, Accrington, UK). The bed bugs were starved for six days prior to the experiment, as starved bed bugs are less likely to aggregate (Romero et al. 2010a).

Interceptor trap catch. The study was conducted in a non-ventilated room at 24-25°C and a 12:12 h (L:D) photoperiod. Each day carbon dioxide (CO_2) at the rate of 100 ml/min was released in the center of the room during the dark cycle using methods described in Singh et al. (2013a) to stimulate bed bug foraging activity (Singh et al. 2013a, Aak et al. 2014).

The bioassay was conducted in plastic arenas (80 × 75 × 5 cm) with bottoms lined with brown paper and a layer of fluoropolymer resin (BioQuip products, Rancho Dominguez, CA, USA) applied to inner walls to prevent bed bugs from escaping. Four interceptors were placed in each arena, with one in each corner. Aged interceptors were used to mimic the condition that interceptors would be in under field conditions. Interceptors were aged prior to the experiment, by placing them in occupied apartments for two weeks and then retrieving them for the experiment. One hundred and fifty bed bugs (40 1^{st} instars, 40 3^{rd}-5^{th} instars, 35 adult males, and 35 adult females) were contained in the center of the arena with a plastic ring (6.4 × 13.3 cm) at one hour into the dark cycle. After one hour conditioning period, the plastic ring was removed. Six arenas were used to provide six replications.

After four days, the number of bed bugs trapped in interceptors and those remaining in each of the arenas were counted by developmental stage and adult sex. Bed bugs not captured were removed from the arenas, while those captured in interceptors were left in place to examine escape rates of captured bed bugs in the next experiment.

Escape of bed bugs from interceptors. The six arenas containing interceptors with trapped bed bugs from the previous experiment were moved to a ventilated room at 23-25°C, 40% RH, and 12:12 h (L:D) photoperiod. During the experiment, CO_2 was released in the same manner as the previous experiment. Eight folded paper harborages (5.1 × 3.3 cm) were placed along the edges of the arena floor (two per side) and another in the center of the arena. The paper harborages were conditioned with bed bug feces and were used in the experiment to stimulate movement and subsequent arrestment (Romero et al. 2009a) of bed bugs escaping from interceptors. Prior to the being used in the assay the harborages had been used in rearing containers for immature bed bugs, thus each harborage contained numerous bed bug feces but no eggs. The number of live and dead bed bugs, in and outside of interceptors and on paper harborages, was recorded by developmental stage and adult sex at 24 h and then every two days for the next ten days. Bed bugs that were unable to crawl when gently prodded were considered dead. After each observation, bed bugs that escaped from interceptors were removed from the arenas.

Effect of marking procedure on bed bug survival

The excess bed bugs collected from one of the m-r-r apartments were divided into two groups: a marked group (22 males, 19 females) and an unmarked group (24 males, 25 females). The marked bed bugs included yellow (10), green (6), red (9) (Apple Barrel, Plaid Enterprises, Inc., Norcross, GA, USA) or white (16) (Folk Art, Plaid Enterprises Inc., Norcross, GA, USA) (Fig. 3.1). A single dab of paint was applied to the top of the thorax-abdomen using a fine bristle paint brush. Once the paint was dry, the marked bed bugs were transferred into a round plastic container (4 × 5 cm). The same day both groups (marked and unmarked) were fed defibrinated rabbit blood as described in the previous laboratory bioassay. The next day, the marked and unmarked bed bugs were placed in plastic arena (80 × 75 × 5 cm) lined with brown paper and a layer of fluoropolymer resin applied to inner walls to prevent the bed bugs from escaping. Eight folded paper harborages were placed along the edges in each arena. The arena was kept in a room at 24 ± 1°C, 40% RH, and a 12:12 h (L:D) photoperiod. Mortality was recorded daily over the next 14 d.

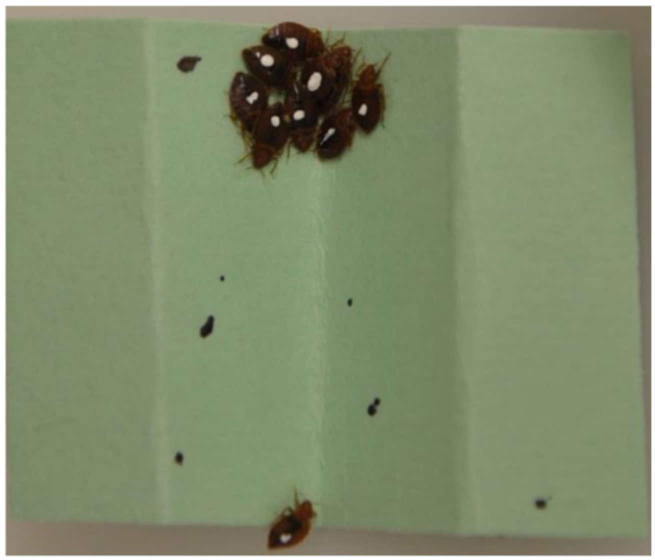

Figure 3.1. Marked bed bugs.

Field Experiments

Selection of study apartments

M-r-r experiments were conducted in bed bug infested apartments located in affordable housing communities in three cities in New Jersey: Irvington, Hackensack, and Newark. In each of the three communities, apartments with large bed bug populations were identified by the housing staff and then visually inspected by three Rutgers researchers to assess the extent of the infestation. A total of six apartments (2 vacant, 4 occupied) were selected for m-r-r experiments. All of the apartments were concrete pre-caste construction with the exception of one apartment (referred to later as apt. #5) which was wood frame construction. The criteria for inclusion of occupied units in the study were: 1) residents were not suffering bite symptoms or being negatively affected by the presence of bed bugs in their apartments, and 2) residents agreed with the study procedures. Neighboring apartments above, below, to both sides, and across the hallway from m-r-r apartments were also inspected and monitored for bed bug activity throughout the study. A general description of the six apartments is provided in Table 3.1 Five of the six apartments were observed for one month between the months of December and February, while one apartment (apt. #6) was observed for five months.

Table 3.1. Overview of the mark-release-recapture apartments.

Site	Apt. #	Apart-ment size	Occupancy status	Mean (min, max) temperature inside apartment (°C)[a]	# of interceptors	Pre-count[b]	Number of bed bugs released by area				
							Bedroom[c]		Living room	Bathroom	Total
I	1	Studio (27 m^2)	Occupied	30 (15-33)	24	305	170 (100:70)[d]	170 (100:70)	170 (100:70)	170 (100:70)	680 (400:280)
I	2	1 BR (45 m^2)	Occupied	25 (19-29)	27	55	92 (52:40)	92 (52:40)	46 (26:20)	46 (26:20)	276 (156:120)
I	3	1 BR (45 m^2)	Occupied	23 (13-27)	29	37	40 (20:20)	0	20 (10:10)	20 (10:10)	80 (40:40)
II	4	1 BR (45 m^2)	Occupied	24 (17-29)	36	105	150 (75:75)	150 (75:75)	150 (75:75)	150 (75:75)	600 (300:300)
II	5	Studio (27 m^2)	Vacant	–[e]	26	191	180[f] (90:90		180 (90:90)	0[g]	360 (180:180)
III	6	1 BR (47 m^2)	Vacant	23 (18-41)	28	575	159 (90:32:37)[h]		159 (90:32:37)	159 (90:32:37)	477 (270:96:111)

[a] Temperature recorded every hour during study period using HOBO data loggers (Pendant temp/light, Onset Computer Corp., Bourne, MA.).

[b] Pre-count for apartments #1, 2, 3, and 6 are based on a 1 d trapping period, apartments #4 and 5 are based on the daily average of a 2 and 4 d trapping period, respectively.

[c] Numbers in first and second columns refer to bed bugs released at the head and foot of the bed respectively.

[d] Numbers in parenthesis refer to adult males:adult females.

[e] Data not available.

[f] In apartments #5 and #6, marked bed bugs of one color were released along base of wall in the bedroom.

[g] No bed bugs were released in the bathroom because it was located less than 2 meters from the apartment entry door.

[h] Numbers in parenthesis refer to large nymphs:adult males:adult females.

Mark-release-recapture procedures at site I. Apartment #1 was located on the 4^{th} floor of an 11-story high-rise, while apartments #2 and #3 were located in a second 11-story high-rise on the same property. Apartments #2 and #3 were located in opposite wings of the building, on the 8^{th} and 9^{th} floors, respectively. All three apartments had a limited amount of furniture and were not cluttered. In apartment #1, the mattress and box spring were still wrapped in the original plastic from the time of purchase. None of the apartments had been treated for bed bugs prior to the study.

Bed bug adults were collected from each apartment over a two day period. Nymphs were not used for marking because they will molt and lose paint marks. Collection methods included: 1) hand removing using featherweight soft forceps (BioQuip Products, Rancho Dominguez, CA, USA), and 2) placement of two un-baited dog bowl traps (Singh et al. 2013a) beneath the bed overnight. The collected bed bugs were placed in plastic containers (4.7 cm height and 5 cm diameter) with paper harborages and held in the laboratory at 22-25^{o}C and natural light conditions. The bed bugs from each of the apartments were marked 24-48 h after collection. Marked bed bugs were held for a total of two to three days without feeding before being released back into the apartment they were collected from. Any marked bed bugs that were unable to crawl when gently prodded were considered dead or moribund. These bugs were removed and replaced with marked bed bugs of the same color. Total mortality and morbidity of marked bed bugs prior to release was $\leq$ 1.5% for all three apartments.

At the time of the initial collection of bed bugs from the m-r-r apartment, interceptors were installed throughout each of the m-r-r apartments to monitor the bed bug activity. Interceptors were placed next to the legs of the bed frame, rather than beneath the legs. This was done to permit movement of marked and unmarked bed bugs to and from the beds. Interceptors were also placed throughout the apartment in the bedroom, living room, closets, bathroom, and kitchen (Table 3.1 and Fig. 3.2a-c). The mean (min, max) distance between the interceptors in the m-r-r apartments was 1.28 (0.30, 2.90) m. Two interceptors were also placed in the hallway outside the apartment on either side of the entry door and were secured to the floor with tape to prevent them from being accidentally moved. Interceptors were installed throughout each of neighboring apartments above, below, to both sides, and across the hallway from each of the m-r-r apartments in a similar manner as the m-r-r apartments. The mean (min, max) number of interceptors in the neighboring apartments was 20 (13, 27).

Marked bed bugs were released into the same apartment from which they were collected, and released by removing harborages from a container with bed bugs of the same color and placing them as a group in one location in the apartment. Any marked bed bugs not resting on a harborage, were gently removed from the container with soft forceps and placed on a paper harborage with the other marked bed bugs of the same color. In all three apartments bed bugs were released at the following locations: 1) on the bed beneath the fitted sheet; 2) in the living room on the floor along the wall; and 3) in the bathroom behind the base of the toilet (to reduce visibility). The number of marked bed bugs released in each apartment as well as the release locations are summarized in Table 3.1 and Fig. 3.2a-c.

Figure 3.2. Apartment diagrams and interceptor locations. Letters a to f refer to apartments #1 to 6, respectively. Circles indicate interceptor trap locations. Colored symbols with an "R" inside, indicate where marked bed bugs of a particular color were released.

The m-r-r apartments were visited on 1, 3, 6, 10, 15, and 29 d after release. During each of these visits interceptors were inspected. Trapped bed bugs were categorized as either small (1^{st} – 2^{nd} instar) or large (3^{rd} – 5^{th} instar) nymphs, and adults were sexed. Captured bed bugs were removed from the interceptors and either destroyed or returned to the laboratory. During each visit, interceptor traps were cleaned and lubricated with talc or replaced with new traps depending upon their conditions. In accordance with the approved IRB protocol bed bug infestations in the three occupied m-r-r apartments were treated at two weeks after the trap catch was recorded.

The neighboring apartments were visited on 3, 6, 10, 15, and 29 d during which time interceptors were inspected and trap catch recorded as described above for the m-r-r apartments. A visual inspection of beds and upholstered furniture was conducted during the final visit.

Mark-release-recapture procedures at site II. Apartment #4 was located on the 3^{rd} floor of a 7-story building and apartment #5 was located on the 4^{th} floor of a 10-story building. The bedroom of apartment #4 was cluttered. The mattress, box spring, bed frame, and built-in headboard were heavily infested. The living room was furnished with a sofa and two upholstered chairs, all with signs of bed bug infestation, but no live bed bugs were observed during visual inspection. The apartment was treated with a liquid residual pyrethroid, Suspend SC (0.03% deltamethrin, Bayer Environmental Science, Montvale, NJ, USA) by the existing pest control contractor less than one hour prior to our initial inspection, however they did not remove the mattress and box spring during their treatment. Adult bed bugs were collected from the mattress and box spring and returned to the laboratory in the same manner as described for apartments at site I. The bed bugs were held for three days prior to marking. Their mortality was 1.1%, suggesting that the bed bugs were either not contacted by the insecticide or were resistant to the pyrethroid insecticide applied. After being marked, they were held in the laboratory for two more days and dead or moribund bed bugs were replaced with freshly marked bed bugs immediately prior to release. The mortality among marked bed bugs was < 1.0% prior to release.

Prior to releasing the marked bed bugs, interceptors were installed in the same manner as described for site I (Table 3.1 and Fig. 3.2d). The mean (min, max) distance between the interceptors in the m-r-r apartments was 1.28 (0.30, 2.90) m. The neighboring apartments of # 4 were also monitored prior to the release of marked bed bugs, in the same manner described for site I. The mean (min, max) number of interceptors per neighboring apartment was 29 (25, 31).

Marked bed bugs were released in the same manner as described for site I (Table 3.1 and Fig.3.2d). The apartment was visited on 1, 3, 7, 14, 21, and 28 d and inspected as described for site I. At 14 d after the trap catch was recorded the apartment was treated in accordance with the approved IRB protocol for occupied units. The neighboring apartments were visited following the same schedule as the m-r-r apartment.

The resident in apartment #5 was evicted the day the m-r-r study began. The apartment was severely cluttered with piles of trash, papers and clothing. Furniture in the apartment was limited to a bed, TV stand, dresser, and recliner. The resident had slept in the recliner for several months prior to the start of the study. Bed bugs were hand collected the same day the resident was evicted. In addition, a CO_2 trap (Singh et al. 2013a) was placed overnight at the recliner to collect more bed bugs. CO_2 was released from the trap at a rate of 200 ml/min between the hours of 10 pm and 8 am. Bed bugs were collected from the CO_2 trap after one day and were held in the laboratory for 24-48 h before being marked. Dead bed bugs were replaced with freshly marked bed bugs immediately prior to release. Mortality of the marked bed bugs prior to release was < 1.0%. Prior to releasing the marked bed bugs, interceptors were installed (Table 3.1 and Fig. 3.2e). The mean (min, max) distance between the interceptors in the m-r-r apartments was 1.16 (0.30, 2.90) m. In addition to the interceptors, a 7.6 cm wide sticky tape barrier was installed across the inside threshold of the entry door to intercept bed bugs traveling at the base of the door. Interceptors were installed in the neighboring apartments six days prior to the release of marked bed bugs, in the same manner described for site I. The mean (min, max) number of interceptors per neighboring apartment was 27 (20, 31).

The CO_2 trap used for the collection of bed bugs remained in place to stimulate bed bug activity in the absence of a host during the monitoring period. The trap was set to release CO_2 at 200-400 ml/min between the hours of 10 pm and 8 am and was turned off at 1, 8-9, 22-23, and 26-28 d to investigate the influence of CO_2 on bed bug movement. The same day the monitors were installed, property management bagged and removed all clutter from the apartment leaving only the bed, reclining chair, wooden dresser, and TV stand.

Marked bed bugs were released in two locations: 1) on the recliner 0.5 m from a CO_2 trap, and 2) along the base of the wall next to the bed (Table 3.1 and Fig. 3.2e). No marked bed bugs were released in the bathroom because it was located immediately adjacent to the entry door and we did not want to promote dispersal so close to the apartment entry. The apartment and its neighbors were visited on 1, 3, 7, 14, 21, 28, and 35 d. The sticky barrier was replaced at least once per week and CO_2 cylinders were replaced as needed to prevent running out before the next visit. At 35 d the apartment was treated for the first time, it had not previously been treated prior to the start of the study.

Mark-release-recapture procedures at site III. Apartment #6 was located on the 5th floor of a 5-story apartment building. The apartment had become vacant 17 d prior to the m-r-r study. Furniture in the apartment was limited to an upholstered chair in the living room along with a small coffee table and a TV. Clothing, boxes, and piles of papers were strewn about the living room. The bedroom had a wooden folding chair and blankets on the floor that the resident slept on. The blankets were heavily infested. Bed bugs were present along the base of the wall less than one meter from where the resident slept. The apartment had not been treated for bed bugs prior to the m-r-r study.

Bed bug adults and large nymphs were hand collected using soft forceps and two CO_2 traps over a two day period. We collected large nymphs at this site because the apartment had been vacant for 17 d prior to collection, thus no more nymphs would molt. They were marked 24-48 h after collection and held for another two days before being released. Dead bed bugs were replaced with freshly marked bed bugs immediately prior to release. Mortality of the marked bed bugs prior to release was <1.5%. One day prior to release, interceptors were installed in the m-r-r apartment and a sticky tape barrier was installed at the entry door as described for apartment #5 (Table 3.1 and Fig. 3.2f). Marked bed bugs were released in the same manner as for site I. Interceptors were also installed in its four neighboring apartments in a similar manner as previously described for site I. The mean (min, max) number of interceptors per neighboring apartment was 30 (27, 33).

The two CO_2 traps, used for the collection of bed bugs were left in place during the post-release monitoring period and maintained in a similar manner as apartment #5. The CO_2 was turned off at 2-4, 6-9, and 18-21 d to investigate the influence of CO_2 on bed bug movement. The apartment was visited daily during the first 12 d, every 3 d for the next 18 d, and then every 5-7 d through 116 d. Interceptors were inspected and maintained as described for site I. On the 24th d the contents of the apartment (furniture, clothing, and debris) were bagged and discarded leaving the apartment empty aside from the monitoring devices. Alpine dust (0.25% dinotefuran, 95% diatomaceous earth dust, Whitmire Micro-Gen Research Laboratories, St. Louis, MO, USA) was applied to the baseboards throughout the apartment. At 71 d, the CO_2 traps were removed from the apartment for the remainder of the study. The interceptors in the neighboring apartments were inspected at 3, 5, 12, 19, and 24 d post-release.

Estimating Population Size

The following conditions are required for estimating populations using m-r-r method (Stradling 1970): 1) the marked bed bugs retain their marks; 2) the marked bed bugs mix thoroughly with the rest of the population; 3) a sample representative of the whole population is taken for marking and for estimation; 4) the population is closed, or rates of immigration and emigration are known; and 5) there are no births or deaths during the period of mixing. Our study did not meet the last two requirements. However, because we were only sampling for a short period of time, we could assume the population size in each apartment was relatively stable.

The bed bug population size in each apartment was estimated using Peterson-Lincoln index (Stradling 1970): $N = M(C+1)/(R+1)$, where N = total population, M =

number of marked bed bugs released, and C = total number in the re-capture sample (marked + unmarked), R = the number of marked individuals in the recapture sample. The variance is calculated by the formula: $V = M^2(C\text{-}R)/((C+1)(R+1))$. Adult stage was used in the population estimates because nymphs were only marked for one of the six m-r-r apartments. The estimated bed bug population is calculated as the estimated number of adults divided by the proportion of adults among all trapped bed bugs.

Statistical Analyses

The effect of marking procedure on bed bug survival was analyzed using a chi-square test. Percentage of bed bugs of various stages or sex trapped in interceptors, recapture rate of marked bed bugs, the small:large nymph ratio at the bed and apartment entry, and the male:female ratio of released and recaptured marked bed bugs were subjected to Student's *t* test. The effect of CO_2 presence on trap catch in a vacant unit was examined using analysis of variance. Correlation analysis was conducted between the number of dispersed and recaptured bed bugs and the number of released bed bugs. All field data analyses were based on interceptor trap catch over 14-15 d prior to treatment with pesticides. All analyses were performed using SAS software version 9.3 (SAS Institute, Cary, NC, USA).

Results

Laboratory bioassays

Reliability of interceptors for estimating bed bug population structure

Among the trapped bed bugs in interceptors, the percentage (mean ± SEM) of 1st instar, 3rd-5th instar, adult males, and adult females was 21.8 ± 2, 22.7 ± 2, 25.7 ± 1, and 29.8 ± 1%, respectively (Table 3.2). There was a significant bias for trapping adult females (t = 2.6; df = 5; $P = 0.046$). The mean percentage of females in the trapped population was 28% more than the percentage of females in the population initially released in the arena, indicating females moved more than adult males and nymphs. The mean percentage of all other stages trapped was similar to the percentages released (1^{st} – 2^{nd} instar: t = -0.7, df = 5, $P = 0.50$; 3^{rd} – 5^{th} instar: t = -0.3, df = 5, $P = 0.81$; males: t = -0.9, df = 5, $P = 0.40$), indicating that the interceptor trap catch is not biased for nymphs and adult males.

Only 1^{st} instar bed bug nymphs escaped from interceptors (Table 3.2). The mean ± SEM percentage of escape in 10 d was 20 ± 6%. Overall, among all stages of bed bugs trapped, the mean ± SEM escape rate in 10 d was 4 ± 1%. The mean ± SEM mortality among bed bugs still trapped in interceptors at 10 d was 99 ± 1%.

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Table 3.2. Trap and escape of bed bugs from interceptors

Rep.	# of bed bugs trapped in interceptors in 4 days					# bed bugs escaped from interceptors within 10 days				
	Adult males	Adult females	1st instar nymphs	3rd-5th instar nymphs	**Total**	Adult males	Adult females	1st instar nymphs	3rd-5th instar nymphs	**Total**
1	14	22	15	13	**64**	0	0	1	0	**1**
2	22	22	18	14	**76**	0	0	3	0	**3**
3	17	20	9	16	**62**	0	0	3	0	**3**
4	19	20	10	20	**69**	0	0	4	0	**4**
5	18	20	20	16	**74**	0	0	4	0	**4**
6	23	26	26	20	**95**	0	0	1	0	**1**
Sum	113	130	98	99	**440**	0	0	16	0	**16**
Mean	19	22	16	17	**73**	0	0	2.7	0	**2.7**

Effect of marking procedure on bed bug survival

Cumulative mortality over 14 d was 24% for both marked and unmarked bed bugs (Fig. 3.3). There was no significant difference in the mortality of marked (14%) and unmarked (13%) males ($\chi^2 = 0.013$; df = 1; $P = 0.91$) or marked (37%) and unmarked (36%) females ($\chi^2 = 0.003$; df = 1; $P = 0.95$). The results indicate that the marking procedure did not have an effect on the survival of the bed bugs. However, female mortality (marked and unmarked) was higher than male mortality (marked and unmarked) ($\chi^2 = 5.13$; df = 1; $P = 0.02$).

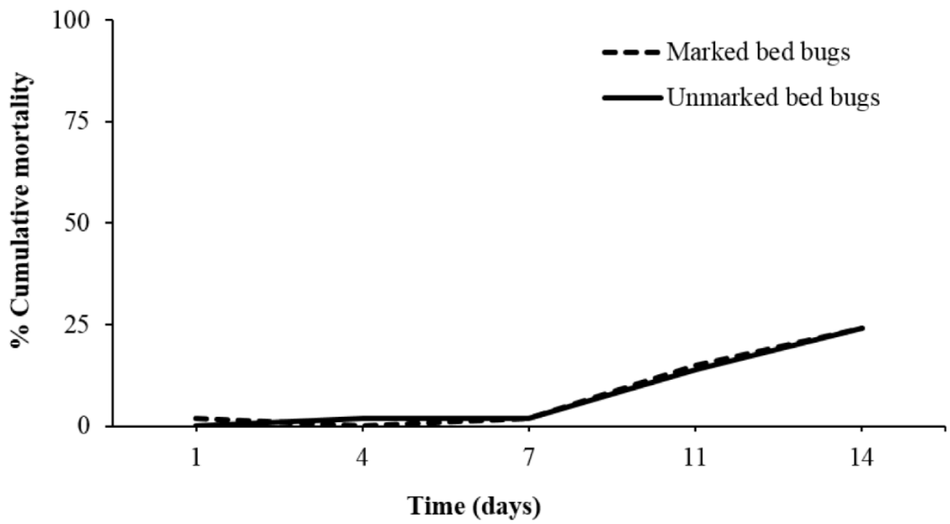

Figure 3.3. Cumulative mortality of marked and unmarked bed bugs under laboratory conditions.

Field Experiments

Movement within apartments

Marked bed bugs were captured in interceptors located at and away from release locations at 24 h post-release in all six m-r-r apartments (Fig. 3.4).

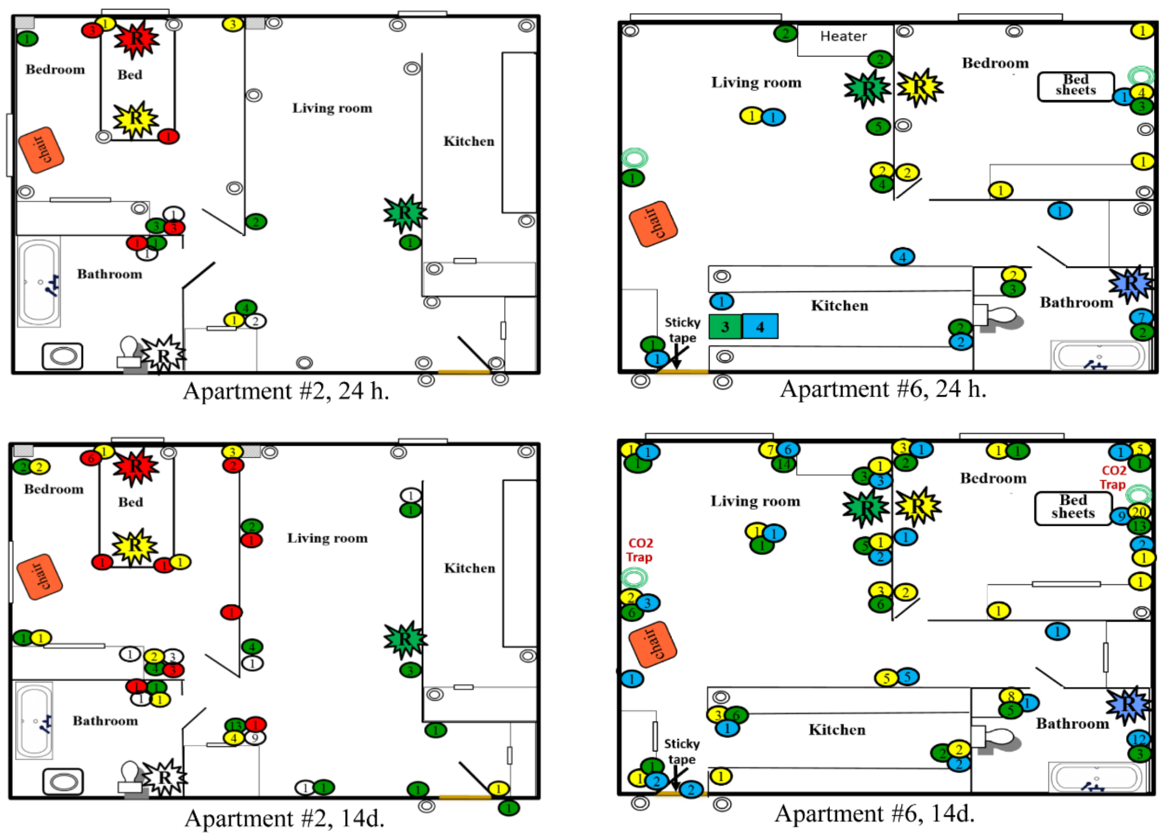

Figure 3.4. Cumulative movement of marked bed bugs within apartments at 24 h and 14 d after release. Two one bedroom apartments (#2 and 6) were selected to illustrate the movement of the marked bed bugs following release. Marked bed bugs moved from their point of release, to a different room in both apartments within 24 h. Numbers in circles represent the number of marked bed bugs of that color trapped in a particular location.

Recapture rates for the marked bed bugs released in the six m-r-r apartments ranged from 6-72% by 14-15 d (Table 3.3). The two vacant apartments had the highest recapture rates (Table 3.3).

Table 3.3. Cumulative recapture rate of marked and released bed bugs over 14-15 days.

Apt.#	Total recapture rate	Recapture rate by stage and adult sex			Recapture rate by release site[a]		
		Large nymphs	Male	Female	Bedroom	Living room	Bathroom
1	40%	–[b]	31%	54%	37%[c]		52%
2	31%	–	25%	38%	18%	76%	37%
3	28%	–	25%	30%	5.0%	60%	40%
4[d]	6%	–	6%	7%	7%	5%	–[e]
5	72%	–	64%	79%	72%		–
6	44%	50%	31%	38%	48%	45%	39%

[a] This rate is the number of bed bugs released in a given room that were recaptured throughout the apartment divided by the number released in that room.

[b] No marked bed bugs were released.

[c] Studio units (#1 and 5) had no distinction between the bedroom and living room.

[d] Resident interfered with study by moving and emptying interceptor traps (apt. #4)..

[e] Resident discarded harborages with marked bed bugs on the day of release (apt. #4).

Females represented 48% ± 2% of the marked adult bed bugs released and 54% ± 1% of the marked adults recaptured. The percentage of recaptured females was significantly higher than the expected percentage ($t = 4.1$; $df = 5$; $P = 0.01$). Therefore, the counts based upon interceptors were biased towards females, suggesting females were more active than males. Similarly, there were significantly more females than males among the 1,810 unmarked adults captured in the m-r-r apartments ($t = 3.56$; df = 5; $P = 0.02$). The mean (min, max) number of marked and unmarked bed bugs captured in interceptors at the entry door over 14-15 d from all six m-r-r apartments was 3.5 (1, 11) and 37.2 (1, 74), respectively. The sticky tape barrier captured 9 marked bed bugs and 248 unmarked bed bugs of all stages in apartment #5, and 2 marked bed bugs and 21 unmarked bed bugs of all stages in apartment #6. These results indicate that bed bug activity is prevalent at apartment entries.

Based on four apartments (#1, 2, 3, and 5) where bed bugs were released directly on the furniture used by the resident to sleep, between 38-67% of the recaptured marked bed bugs were in interceptors located at least 2.5 m from the host sleeping area. Among the three one bedroom apartments (#2, 3, and 6), recapture rates were significantly greater in the room of release than the non-release rooms, when marked bed bugs were released in either the bedroom ($F = 9.0$; df 3, 8; $P = 0.01$) or living room ($F = 7.9$; df = 3, 8; $P = 0.01$), but not the bathroom ($F = 2.8$; df = 3, 8; $P = 0.11$) (Table 3.4). Of those released in the bedroom of these three apartments, a mean of 42 ± 7.5% of the marked bed bugs were recaptured outside of the bedroom. The mean ± SEM percentage of unmarked bed bugs captured at the bed, in the bedroom away from the bed, in the living room, in the bathroom, and in other areas (i.e. hallway, kitchen, entry door) was 15 ± 5, 41 ± 10, 30 ± 9, 3 ± 1, and 11 ± 6%, respectively. These results demonstrate the extensive movement of bed bugs within apartments.

Table 3.4. Movement of marked bed bugs within apartments based on 14-15 day cumulative trap catch.

Apt.#	Total number of recaptured marked bed bugs	Percentage of marked bed bugs recaptured by location based upon point of release											
		Released in bedroom				Released in living room				Released in bathroom			
		Bed-room	Living room	Bath-room	Other[a]	Bed-room	Living room	Bath-room	Other	Bed-room	Living room	Bath-room	Other
2	85	73	18	6	3	23	71	3	3	24	64	6	6
3	22	50	0	0	50	0	75	0	25	38	25	12	25
6	208	51	21	12	16	24	51	11	14	27	33	22	18
Mean	**105**	**58.0**	**13.0**	**6.0**	**23**	**15.7**	**65.7**	**4.7**	**14.0**	**29.7**	**40.7**	**13.3**	**16.3**

[a] Other areas include the apartment entry door, kitchen, hallway, and closets.

A total of 4,076 (4,062 unmarked and 14 marked) bed bugs were captured in 20 of the 24 neighboring apartments over 14-15 d. Fifteen of the 20 apartments were one bedroom units and the rest were studio units. The median (min, max) bed bug count in the 20 infested apartments was 6.5 (1-3,162) bed bugs over 14-15 d. Bed bugs were captured outside of the bedroom and living room in 18 of 20 apartments. In the three one bedroom apartments with sufficient adult bed bug counts (> 10) for analysis, the mean percentage of females captured in the bedroom and living room compared to areas outside the bedroom and living room was 0.71 ± 0.08 and 0.55 ± 0.12, respectively. They were not significantly different (t = 0.85; df = 2; P = 0.48).

Movement between apartments

A total of 14 marked bed bugs (7 nymphs, 6 females, and 1 male) were recaptured in neighboring apartments of four m-r-r apartments over 14-15 d (Table.3.5). Among these four m-r-r apartments, at least one of the dispersing bed bugs in each apartment was released at the host sleeping area. Marked bed bugs of three different colors dispersed from apartment #6. These results indicate that bed bugs from any room within an apartment, even those located at host sleeping sites, have the potential to disperse to neighboring apartments. The highest dispersal rate (the percentage of marked and recaptured bed bugs in neighboring apartments divided by total recaptured marked bed bugs) at 14-15 d was 5.0%. The number of recaptured bed bugs in neighboring units was not correlated with the number of released bed bugs (F = 0.96; df = 1, 4; P = 0.38). The fact that one vacant apartment (#6) had the highest active dispersal rate and the other vacant apartment (#5) had no active dispersal indicates that vacancy is not necessarily correlated to bed bug dispersal.

Table 3.5. Active dispersal of bed bugs revealed from m-r-r technique over 14-15 days.

Apt. #	# of unmarked/ marked bed bugs trapped in mark-release apt	Number of unmarked/marked bed bugs captured in apartments surrounding the m-r-r apartments					% dispersal rate[a]	Areas where Marked bed bugs dispersed from	Areas where marked bed bugs were recaptured in neighboring apartments
		Adjacent to the right	Adjacent to the left	Across hall	Above	Below			
1	3,090/280	8/1	3/0	6/0	120/0	37/0	0.4	Bed	Bedroom
2	220/85	3162/1	na	Na	1/0	1/0	1.2	Bed	Kitchen
3	288/22	0/0	0/0	Na	0/0	7/0	0	None	None
4	1,020/30	575/1	87/0	na.	1/0	0/0	3.2	Bed	Kitchen
5	11,315/258	2/0	na	2/0	5/0	1/0	0	None	None
6	1,924/208	26/2	27/4	7/4	na	3/1	5.0	Bedroom, living room, bathroom	Kitchen, hall, living room

[a] Dispersal rate is calculated as the total number of marked bed bugs recaptured in neighboring apartments divided by the total number of marked bed bugs recaptured.

Between 16-28 d, marked bed bugs dispersed from two m-r-r apartments (#4 and 5), one of which (apt. #5) did not show active dispersal during the first 15 d. Each of these two apartments had two marked adult females captured in their neighboring apartments. It should be noted that apartment #4 was treated at 15 d. Additionally, a marked adult female (green) was captured in an interceptor in the hallway outside of apartment #2. Although active dispersal was not recorded for apartment #3, two marked adult females, one released at the bed (yellow) and one released in the living room (green), were captured in interceptors at the entry door of this apartment. Over 28-32 d a total of 12 marked bed bugs (11 females, 1 male) were recaptured in ten neighboring apartments. All of these neighboring apartments had bed bug activity. Horizontal dispersal of marked bed bugs to a neighboring apartment on the same floor occurred among all four m-r-r apartments where dispersal was recorded within 28-32 d. Marked bed bugs were found in two of the three apartments that were across the hallway from a m-r-r unit. Vertical dispersal, on the other hand, was observed from two out of the five m-r-r apartments.

Longevity in the absence of a host

In apartment #6, the mean (min, max) daily temperature inside the apartment during the study was 23 (18, 41)^{o}C. After release, marked large nymphs, adult females, and adult males continued to be captured up to 57, 113, and 134 d after vacancy, respectively.

Unfed, unmarked small nymphs were no longer captured in interceptors after 134 d. Other stages of unfed bed bugs continued to be found in interceptors after 134 d. When the experiment was terminated at 155 d, 16 unmarked bed bugs were captured in interceptors over a 7 d trapping period. Of these, seven were large nymphs and nine were adults (8 female, 1 male). These results demonstrate that young nymphs were able to survive at least 4.5 mo and all other stages over 5 mo in the absence of a host. The small:large nymph ratio declined sharply after 56 d (Fig. 3.5). The sudden decline suggests that most small nymphs survived starvation for 52-69 d and large nymphs are more tolerant to starvation than small nymphs.

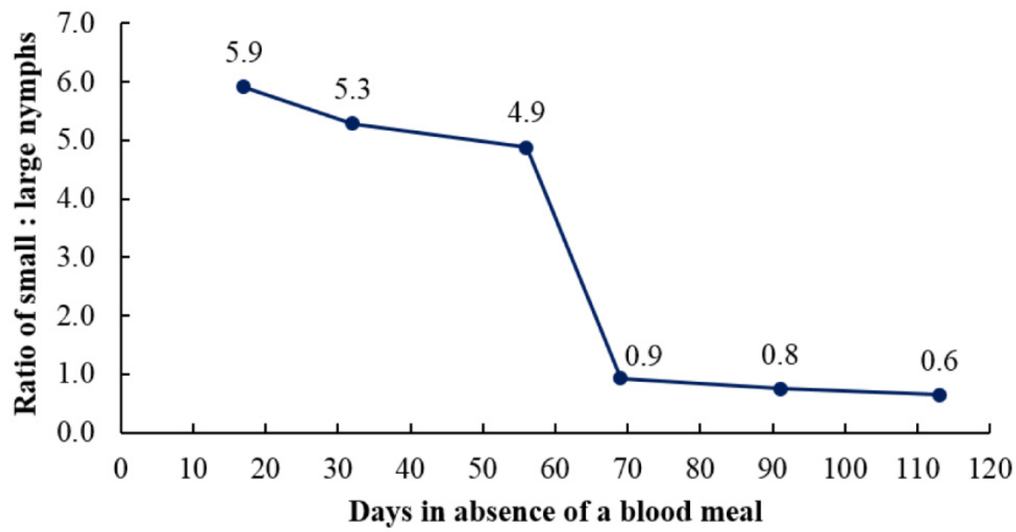

Figure 3.5. Ratio of unmarked small:large nymphs over time in the absence of a host in a vacant apartment.

Estimating population size

Four apartments (#1, 2, 5, and 6) were selected to estimate population size. The other two apartments (#3 and 4) had too few recaptured marked bed bugs (4 and 7 bugs) during 4-7 d and therefore were excluded from population estimation analysis.

We used data where the percentage of marked adults became stable (4-7 d) to estimate bed bug populations. The percentage of marked bed bugs in the interceptors was high during the first 3 d after release and became relatively stable until 10 d (Fig. 3.6).

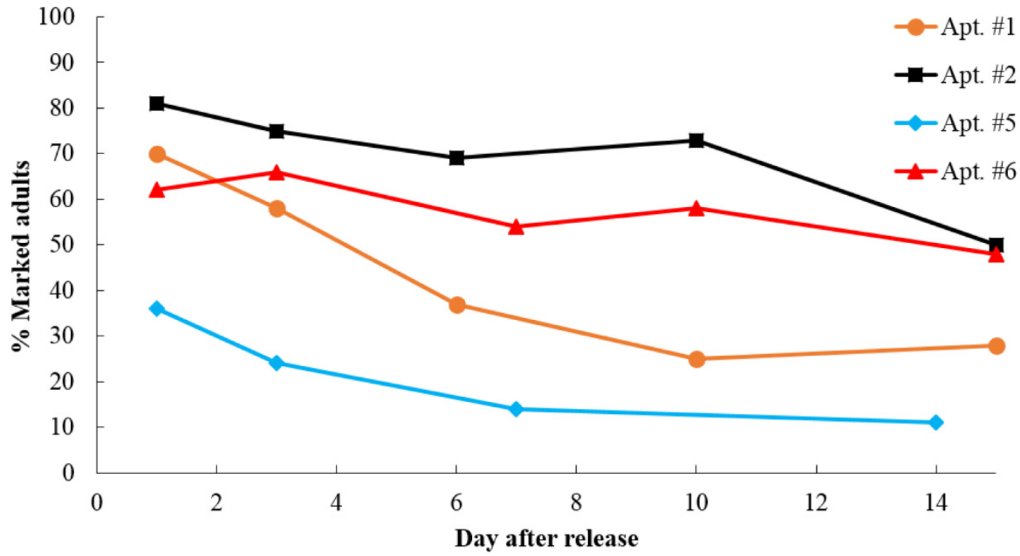

Figure 3.6. Dynamics of the ratio of marked adult bed bugs over all adult bed bugs captured from interceptors.

The initial high percentage of marked adult bed bugs was expected, as the released bed bugs might need a few days to acclimate. The M in the population estimate formula (N = M(C+1)/(R+1) was estimated as the number of bed bugs initially released minus the recaptured marked bed bugs during the first three days because these bed bugs were no longer present. The population estimates for each of the four apartments are summarized in Table 3.6.

Table 3.6. Bed bug population estimation based on m-r-r technique.

Apt. #	Trapping period	Total # of marked adult bed bugs existed at 4 d[1]	Proportion of marked adult bed bugs[2]	Estimated total adults	Proportion of adults in unmarked bed bugs	Estimated total population	Standard deviation
1	4 to 7 d	505	0.3778	1,337	0.09354	14,291	1,578
2	4 to 6 d	226	0.7059	320	0.13158	2,433	381
5	4 to 7 d	177	0.1396	1,268	0.10304	12,305	1,630
6	4 to 7 d	173	0.5476	316	0.02794	11,306	1,586

[1] This is the number of bed bugs initially released minus the recaptured marked bed bugs during the first three days.

[2] This is the (R+1)/(C+1) used in the formula for population estimation.

Once the estimated population size is available, it is possible to estimate what percentage of bed bugs from an apartment can be caught in an interceptor during one day. We used the mean daily counts per interceptor during 4-7 d from four interceptors installed in the area used by the host to sleep during the night. We selected interceptors in these locations to demonstrate how effective interceptors are because this is where interceptors are typically placed by users. Daily trap catch data were only available in one of the two vacant apartments (#6) over the 4-7 d period. During this time CO_2 was released for 1 d and then turned off during the other days. When CO_2 was released, the estimated percentage of bed bugs trapped per interceptor, per day was 0.55%, and when CO_2 was absent it was 0.11 ± 0.02%. Therefore, adding a CO_2 source can greatly increase the trap catch. In the two occupied apartments (#1 and 2), 0.07 and 0.61% bed bugs were trapped per interceptor per day.

The effect of a CO_2 source on interceptor trap catch was further demonstrated in apartment #6. The daily total catch from 28 interceptors were recorded when CO_2 was not available during 7-9 d and when CO_2 was released daily at 200-400 ml/min for 10 h per day during 10-12 d. The mean daily total catch was 29 ± 7 and 111 ± 26, respectively. They were significantly different ($F = 9.6$; df = 1, 4; $P = 0.04$). The mean trap catch when CO_2 was released was 3.8 × higher than when no CO_2 was released.

Discussion

Placement of pitfall-style interceptor traps throughout apartments in conjunction with a m-r-r method, proved effective for investigating the activity of *C. lectularius* under field conditions. Our results demonstrate that bed bugs of all developmental stages travel extensively within and between apartments within a building. The dispersal rate of marked bed bugs to neighboring apartments was not correlated to the number of bed bugs released. Starved bed bugs can survive for more than five months under field conditions. These findings provide new insights into the behavioral ecology of bed bugs within infested apartments.

Wang et al. (2009, 2010) showed that over 55% of the bed bugs captured in traps placed under furniture legs were traveling to the beds and upholstered furniture rather than originating from the host sleeping areas. Our results confirm that bed bugs travel extensively within infested apartments in areas away from host sleeping and resting sites. Within 24 h of release, marked bed bugs were captured both at and away from host sleeping/resting areas regardless of their release location in all five m-r-r apartments. Over the course of 14-15 d, between 39-67% of the marked bed bugs released on the furniture where the resident slept during the night traveled at least 2.5 m from the host sleeping area before being captured and 42% of the marked bed bugs released in bedrooms were recaptured outside of the bedroom. The movement of marked bed bugs away from host sleeping areas demonstrates the extensive movement of bed bugs within infested apartments.

It is possible that the movement of the marked bed bugs observed in our study included an artifact created by our experimental design, which involved relocating many of the marked bed bugs to areas other than where they were collected. However, the unmarked bed bugs captured in interceptors represents the natural activity of the population in the m-r-r apartments. Among the unmarked bed bugs captured, 14% were found in interceptors located in bathrooms, kitchens, hallway closets, and door entry areas. In a case study of two occupied homes, bed bugs were marked in situ at resting locations (Potter et al. 2013b). Similar to our results, marked bed bugs moved from host sleeping areas to non-sleeping areas, from non-sleeping areas to different non-sleeping areas, from non-sleeping areas to host sleeping areas, and were recaptured up to nine meters away from their original resting locations. The authors concluded that bed bugs actively move throughout infested dwellings (Potter et al. 2013b).

The active dispersal of bed bugs from an infested apartment to neighboring apartments has long been suspected (Pfiester et al. 2009b, How and Lee 2010, Wang et al. 2010, Booth et al. 2012, Naylor 2012, Lehnert 2013, Saenz et al. 2013), but never proven. Using the m-r-r technique, we were able to definitively demonstrate active dispersal of bed bugs between apartments. Active dispersal to one or more neighboring apartments was confirmed among five of the six m-r-r apartments. Active dispersal occurred from both vacant as well as occupied apartments and was not correlated the number of marked bed bugs released. Moreover, the highest, and one of the lowest, active dispersal rates were observed in the two vacant apartments. The wide variability in the degree of active dispersal among the m-r-r apartments indicated that factors other than infestation level and host availability affect the active dispersal rate.

Active dispersal of bed bugs between apartments has been used as an explanation for infestation clusters and the spread of bed bugs in multi-occupancy buildings (Pinto et al. 2007, Wang et al. 2010, Naylor 2012). For example, Doggett and Russell (2008) documented the spread of bed bugs from 1 to 68 living units in a high rise housing facility in just 25 mo. Among the infested units 85% shared a common wall, ceiling, floor, or were across the hallway from another unit with bed bugs (Doggett and Russell 2008). In our study, among the six m-r-r apartments 83% of the 24 neighboring apartments had bed bug activity. A number of our findings suggest that active dispersal may be the primary cause for these infestations: 1) 92% (11 of 12) of the marked adults recovered in neighboring apartments were females; 2) marked bed bugs were found in 50% of the 20 neighboring infested apartments; 3) marked bed bugs dispersed from the m-r-r apartment to apartments in all directions (above, below, adjacent, and across the hallway); and 4) the m-r-r units had higher population levels than the neighboring infested units except for two apartments. Current recommendations for neighboring unit inspections in multi-occupancy dwellings include the units above, below, and adjacent to the known infested unit (NPMA 2011). However, based upon our findings we recommend expanding the scope of neighboring unit inspections to include units located across the hallway from known infestations.

Recent studies have used aggregation behavior to draw conclusions about dispersal (Pfiester et al. 2009b, Naylor 2012, Lehnert 2013). A limitation of this approach is that it doesn't consider the temporal activity of the bed bugs moving between aggregations and resting sites. In contrast, our approach relies upon intercepting the movement of bed bugs (marked and unmarked) as they travel within the infested dwelling, over time. However, there are also limitations associated with this approach: 1) trapped bed bugs are prevented from reaching their destination and therefore their potential to spread is reduced; 2) because bed bugs were not marked in situ within individual aggregations, we altered the natural distribution and are also likely to have increased their movement.

While our study does not answer the question as to why bed bugs are dispersing, it does provide important insight that can serve as the basis for future study on dispersal mechanisms. For example, it has been suggested that adult females may disperse to avoid the deleterious effects associated with repeated traumatic insemination by adult males (Stutt and Siva-Jothy 2001, Pfiester et al. 2009a and b, How and Lee 2010) and in doing so, can expand the population range and create new infestation sites (Pfiester et al. 2009b, Booth et al. 2012). Pfiester et al. (2009b) also suggested that following female dispersal, male bed bugs may also begin to disperse in search of females. Laboratory bioassays conducted by Naylor (2012) demonstrated that adult male and female bed bugs disperse in equal numbers, a finding which was supported by observations that were made at a single field site. However, we found among marked and unmarked adults, a greater number of females were captured in interceptors, and nine of the ten marked adults that actively dispersed from m-r-r apartments to neighboring apartments were females, demonstrating that females are more active and travel farther than males. Whether or not female dispersal is based upon avoidance of males is unclear.

Another commonly held belief is that bed bugs disperse as infestation levels increase (Pinto et al. 2007, Naylor 2012, Potter et al. 2013b). Potter et al. (2013b) observed dispersal of marked bed bugs away from their original resting sites, but pointed out that the field sites had well established infestations and that the degree of movement might differ with smaller populations. Naylor (2012) conducted extensive laboratory experiments on bed bug dispersal and observed bed bug infestations in four buildings concluding that bed bug aggregations were rarely located more than 2.3 m away from where the host slept, particularly in populations of fewer than 100 bed bugs, and that population density within harborages seems to be the main driving force for dispersal. In our study there was no relationship between the active dispersal rate of marked bed bugs to neighboring apartments and the number of marked bed bugs released in the m-r-r apartments. These results suggest that infestation level alone is not responsible for dispersal. Factors such as the amount of clutter and resident behavior may also have affected the dispersal. However, our sample size was not large enough to analyze these relationships.

One possible explanation for the extensive movement of bed bugs away from host feeding sites could be that the bed bugs have become lost and are unable to locate the host at which time they begin to randomly forage in search of a host. Bed bugs orient towards host cues (primarily CO_2 and heat) over a relatively short distance up to 2 m (Marx 1955, Anderson et al. 2009, Singh et al. 2012) in order to obtain a blood meal. This could explain why Naylor (2012) rarely found aggregations located more than 2.3 m away from where the host slept unless infestation levels were severe. It is possible that bed bugs located outside this range have difficulty locating the host. In bed bugs, a negative correlation exists between starvation duration and aggregation behavior (Reinhardt and Siva-Jothy 2007, Olsen et al. 2009, Pfiester et al. 2009b) and hungry bed bugs have been reported to travel up to 20 m to find a host (Kemper 1936, Usinger 1966). Movement of insects away from their harborage sites can increase with hunger, and this may also promote formation of new harborage sites (Griffiths 1980, Bell 1990). Kemper (1936) suggested that for bed bugs, random appetitive searching is important and found that individuals unable to locate a blood meal will continue to forage all night. Reis and Miller (2011) also demonstrated continued and prolonged foraging among bed bugs that could not access a blood meal in a laboratory bioassay. Potter et al. (2013b) recovered marked bed bugs in interceptor traps up to 9 m from where they had been resting 7 d earlier. In our study it was impossible to determine the exact distance that marked bed bugs traveled, however large nymphs and adult females traveled at least 12 m, based upon the shortest path from the point of release to point of capture in apartments across the hallway. Thus it is possible that bed bugs captured away from host sleeping areas in our study represent bed bugs that were unable to locate the host and continued to forage in a random manner in search of a food source, as suggested by Kemper (1936). We did not specifically record the feeding status of the bed bugs captured in interceptors, however based upon our observations, little to no blood remained in the digestive tract of most bed bugs captured in interceptors and capture of engorged individuals was rare.

For an ectoparasite such as the bed bug, that is capable of surviving long periods (several months or more) without feeding, having a portion of the population "searching" for food could serve as an important mode of dispersal. This might also explain how bed bugs locate different host sleeping and resting sites within a home (i.e. multiple bedrooms, living room, family room, finished basements). More broadly dispersed populations within a single dwelling would also be harder to eliminate compared to populations that are limited to a single bed or bedroom, and in multi-occupancy dwellings would promote spread to other living units.

Previous studies examined bed bug starvation tolerance at various temperatures (Kemper 1930, Johnson 1941, Omori 1941, Polanco et al. 2011c). Early studies by Kemper (1930) and Johnson (1941) reported first instars surviving 84 and 210-213 d in the absence of a blood meal at 22 and 7°C, respectively. At 13°C a single starved adult female survived 562-572 d (Kemper 1930). More recently, under laboratory conditions of 26.1-26.5°C, the maximum survival times for starved first instar nymphs, adult males, and adult females were 38, 106, and 99 d, respectively (Polanco 2011c). We continued to capture starved 1st or 2nd instar nymphs in interceptors up to 134 d and all other stages and adults of both sexes were still being captured at 155 d, when the study was terminated. The mean temperature over the 155 d was 23^{o}C, however the temperature fluctuated widely between 18-41^{o}C throughout the study, climbing during the day due to solar radiation from a southerly exposure to the sun through windows that had no curtains. Under a more stable temperature range as is expected in infested dwellings, survival times may be even longer than what we observed. The large population size in the apartment could also explain the long survival times observed. The greater the initial population size, the higher the number of bed bugs that are likely to survive for longer periods of starvation. Additionally, the pesticide resistance profile for the bed bugs in our study is unknown, which could either increase or decrease the survival times observed.

Our laboratory assays show that interceptors can be used to estimate bed bug population structure with slight bias toward females. Only first instar nymphs were able to escape following capture in interceptors. The high mortality rates among trapped bed bugs is similar to what is seen under field conditions (personal observation) and may be the result of desiccation of the exposed bed bugs in the open well of the interceptors. It is possible that the mean escape rate for 1st instar nymphs would have been less had new interceptors, or a fresh layer of talc been applied to the used interceptors. However, our results with used interceptors are more representative of what would be expected under field conditions.

Interceptors were demonstrated to be an effective and simple method for detecting bed bugs (Wang et al. 2011, Cooper et al. 2014, 2015a). They are placed under furniture legs and checked 7-14 d later. Knowing what percentage of the bed bug population will be trapped during a 7 or 14 d period will help determine the population size and the optimum number of interceptors to be installed for detecting bed bugs. Based on this study, an interceptor placed at the bed or sofa caught only 0.11-0.61% of the bed bug population. It should be noted that the interceptors were placed beside the legs of furniture in this study due to the experiment design. In field practice, interceptors are typically placed directly under the furniture legs, unless they do not fit into the interceptors. In occupied apartments, the percent of the bed bug population being captured by an interceptor can vary greatly. We showed in vacant units, adding a CO_2 source will increase trap catch by 3.8 times. Increasing the number of interceptors or placement period will provide a better estimate about the presence of bed bugs and their distribution.

Conclusion

M-r-r technique and the use of pitfall-style interceptors are effective methods for studying bed bug movement under field conditions and to estimate bed bug populations. Nymphs and adult bed bugs of both sexes are very mobile and travel extensively throughout apartments. Bed bugs have the ability to disperse from occupied and vacant apartments to neighboring apartments. Bed bugs can survive at least 4.5 months of starvation at field conditions. These findings have important implications on bed bug management and eradication programs. Movement of bed bugs away from predictable locations such as beds and upholstered furniture within apartments may complicate control efforts, making it more difficult to eradicate bed bugs and determine when infestations have been eliminated. The active dispersal of bed bugs between apartments suggests inspecting surrounding units, including apartments across the hallway from known infestations, is necessary.

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CHAPTER FOUR

Effects of Various Interventions, Including Mass Trapping with Passive Pitfall Traps, on Low-level Bed Bug Populations in Apartments

Abstract

Two experiments were conducted to evaluate the effects of various interventions on low-level bed bug, *Cimex lectularius* L., populations in occupied apartments. The first experiment was conducted in occupied apartments under three intervention conditions: never treated (Group I), recently treated with no further treatment (Group II), and recently treated with continued treatment (Group III). Each apartment was monitored with pitfall-style traps (interceptors) installed at beds and upholstered furniture (sleeping and resting areas) along with approximately 18 additional interceptors throughout the apartment. The traps were inspected every 2 wk. After 22 wk, bed bugs had been eliminated (zero trap catch for 8 consecutive wk and none detected in visual inspections) in 96, 87, and 100% of the apartments in Groups I, II, and III, respectively. The second experiment investigated the impact of interceptors as a control measure in apartments with low-level infestations. In the treatment group, interceptors were continuously installed at and away from sleeping and resting areas and were inspected every 2 wk for 16 wk. In the control group, interceptors were placed in a similar fashion as the treatment group but were only placed during 6-8 and 14-16 wk to obtain bed bug counts. Bed bug counts were significantly lower at 8 wk in the treatment group than in the control group. At 16 wk, bed bugs were eliminated in 50% of the apartments in the treatment group. The implications of our results in the development of bed bug management strategies and monitoring protocols are discussed.

Introduction

The recent resurgence of bed bugs, *Cimex lectularius* L. and *Cimex hemipterus* F., has been global in nature (Davies et al. 2012, Doggett et al. 2012, Potter et al. 2013), creating economic (Doggett et al. 2012), social (Eddy and Jones 2011, Aultman 2012), and public health (Goddard and de Shazo 2009, Aultman 2012, Doggett et al. 2012, Susser et al. 2012) challenges, as bed bugs spread throughout communities. Failure to recognize or report the presence of bed bugs promotes the establishment of infestations that are more costly and difficult to eliminate (Wang et al. 2010, Singh et al. 2013, Stedfast and Miller 2014, Cooper et al. 2015a). In a field study conducted by Singh et al. (2013), infestations in apartments with initial bed bug counts below 30 were eliminated within 3.5 mo, while those with initial counts over 30 continued to persist beyond 5.5 mo, in spite of repeated treatments. Other field studies have demonstrated that bed bug populations can usually be reduced by more than 90%; however, it is not uncommon for small numbers of bed bugs to persist even after repeated treatments (Potter et al. 2006, 2008; Moore and Miller 2009, Wang et al. 2009, 2013; Potter et al. 2012). Reducing but not eliminating infestations can lead to chronic infestations. Installation of passive pitfall-style traps (interceptors) at, and away from host sleeping and resting areas, is effective for monitoring low-level bed bug activity (Cooper et al. 2014) and can prevent the premature termination of treatments in apartments where bed bugs are present in low numbers but are not detected at host sleeping and resting areas (Cooper et al. 2015a).

Bed bugs exist in small numbers when they are first introduced into a new environment and just prior to the eradication of an infestation (Booth et al. 2012). The success of bed bugs in becoming established following a new introduction or becoming re-established after having populations reduced to very low levels has not been examined. While it is generally agreed upon that light infestations are more easily controlled and less likely to spread (Pinto et al. 2007), the dynamics of low-level infestations are poorly understood. In this paper, two experiments were conducted to investigate the effect of various interventions in apartments with low-level bed bug populations. The first experiment evaluated the dynamics of low-level bed bug populations in apartments with or without treatments. The second experiment investigated the impact of interceptors as a control measure in apartments with low-level infestations.

Materials and Methods

Experiment I. Trap Catch in Untreated and Treated Apartments with Low-level Infestations

The purpose of this experiment was to study the dynamics of low-level bed bug (≤ 10 based on trap counts) populations (*C. lectularius*) in apartments with or without treatments. The apartments were divided into three groups - I: never treated, II: recently treated with no further treatment, III: recently treated with continued treatment. The experiment was conducted in one-bedroom apartments (47 m^2) in an affordable housing community occupied by elderly (> 62 yr old) and disabled residents located in Newark, NJ. This study protocol (# E11-766) received approval from Rutgers University Institutional Review Board (IRB).

Group I: Apartments that had not been treated for bed bugs within the previous two years were used. Climbup® insect interceptors (Susan McKnight, Inc., Memphis, TN), hereafter referred to as interceptors or traps, were installed at 0 wk under the legs of beds and upholstered furniture or immediately adjacent to the furniture, if placement under legs was not feasible. An additional 17-18 interceptors were placed throughout each apartment. Figure 4.1 shows the typical location of traps in apartments. The mean (min, max) number of interceptors placed per apartment was 28 (21, 38). Interceptors were

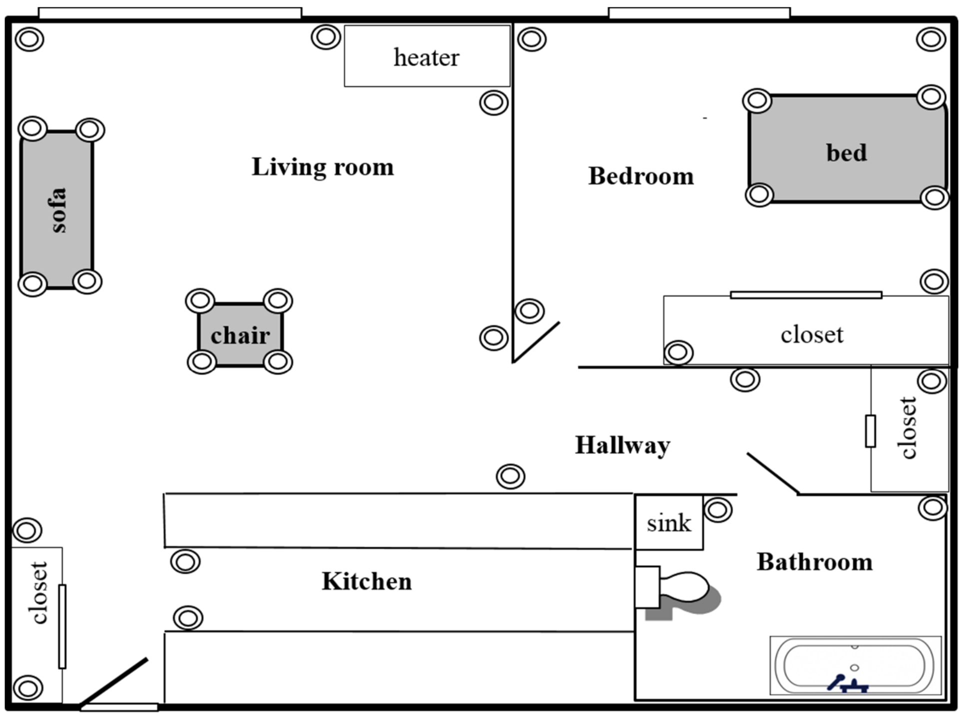

Figure 4.1. Typical layout of interceptors in each apartment in Experiment I. Double circles are interceptors.

inspected for the presence of bed bugs 14 d later. Apartments with a total trap catch of 1-10 bed bugs were included. None of the residents were aware that they had existing bed bug activity. Residents' approval was obtained prior to the study. Residents were asked not to apply any insecticides in their apartments during the study. A total of 23 apartments were identified. No corrective actions (except installation of traps) were taken to control the existing bed bugs. Each apartment was visited by 2-3 Rutgers researchers every 14 d until no bed bugs were captured in any of the interceptors for eight consecutive weeks at which time, a visual inspection of the bed and upholstered furniture was conducted. If bed bugs were detected during the visual inspection, the time was set back to 0 wk and the process was repeated until the elimination criterion was achieved. During each visit, interceptors were inspected for bed bugs, then cleaned and lubricated with talc or replaced with new traps, depending upon their conditions.

Groups II and III: Apartments that were recently treated by an independent professional pest control company for a bed bug infestation were included. The pest control company was blinded from the treatments and the objectives of the experiment. The initial treatment of these apartments by the pest control company included the following: 1) vacuuming visible bed bugs, 2) application of steam to furniture and baseboards, 3) encasing of mattresses and box springs with bed bug encasements (Allerzip® Protect-A-Bed®, Northbrook, IL, USA), 4) installation of interceptors under the legs of beds and upholstered furniture, and 5) spot application of 0.03% lambda-cyhalothrin (Demand® CS, Syngenta Crop Protection, LLC, Greensboro, NC) along baseboards throughout the apartment. Following the initial treatment, the apartments were inspected by the pest control vendor every 14 d, and additional treatments made as necessary, at the technicians discretion, using one or more of the following methods: 1) vacuuming visible bed bugs, 2) application of steam to visible bed bugs, and 3) re-application of a pesticide using 0.05% chlorfenapyr (Phantom SC, BASF Corporation, Durham, NC) to baseboards of the apartments. Follow-up visits continued until no bed bugs were found based upon all of the following: 1) visual inspection, 2) trap catch, and 3) the resident indicated that they had not seen any bed bug activity since the previous visit.

Within one week of the termination of the treatment program, we installed interceptors throughout 67 apartments in the same manner described for Group I. None of the residents were aware that they still had existing bed bug activity. The mean (min, max) number of interceptors placed per apartment was 29 (22, 34). Interceptors were inspected for the presence of bed bugs 14 d later. With the exception of two apartments, whose residents requested their apartment to be in Group III, apartments with a trap catch of 1-10 bed bugs were randomly placed into one of two groups: Group II - no further treatment (23 apartments) or Group III - continued treatment (21 apartments). Apartments in both groups were inspected by Rutgers researches every 14 d until bed bugs were eliminated based on the same evaluation methods as Group I. Apartments in Group III were visited every two weeks by the professional pest control vendor using similar methods as their previous follow-up visits.

In all treatment groups, if the bed bug count increased to 20 or more bugs at any time, the apartment was discontinued from the experiment according to the IRB protocol, and property management was notified so the apartment could be scheduled for treatment. Apartments discontinued from the study were included in data analysis until the time they were discontinued. All of the apartments received a final inspection at 9-12 mo post-elimination. The inspection included monitoring the apartment with interceptors for 14 d followed by a visual inspection of the sleeping and resting areas.

Experiment II. Impact of Interceptors on Low-Level Bed Bug Infestations

Based upon the results of the previous experiment, we investigated if interceptors placed throughout the apartments were contributing to the decline of *C. lectularius* counts and eventual elimination of infestations in apartments with low bed bug counts. The experiment was conducted in an affordable housing community occupied by elderly (> 62 yr old) and disabled residents in Irvington, NJ. Two 11-story apartment buildings were inspected for bed bugs using a combination of visual inspection and placing interceptors. The visual inspection was brief (5-10 min with two people) and limited to beds and upholstered furniture. Interceptors were placed under the legs of beds and upholstered furniture and checked for bed bugs 14 d later. Apartments meeting the following conditions were included: 1) total count of 1-10 bed bugs based upon trap catch and visual inspection count, 2) residents indicated that they were not emotionally upset about the bed bug activity and they did not suffer bed bug bite symptoms and agreed to participate in the study, and 3) residents agreed not to apply any insecticides in their apartments during the study. This study protocol (# E11-766) received approval from Rutgers University IRB.

A total of 36 apartments were used (6 one bedroom and 30 studio apartments). They were randomly divided into two similar groups (18 apartments per group) based upon total bed bug counts and apartment type (one bedroom or studio). Residents were asked whether or not they were aware of the bed bug activity in their apartments. The treatment group had interceptors continuously present both at sleeping and resting areas and along room perimeters throughout the apartments. A mean (min, max) of 22 (13, 35) interceptors were installed in each apartment and then inspected every 14 d for 16 wk. In the control group, interceptors were only present between 6-8 wk and between 4-16 wk in order to obtain bed bug counts at the midpoint (8 wk) and endpoint (16 wk) of the study. A mean (min, max) of 23 (16, 33) interceptors were installed in each apartment. A thorough visual inspection of all furniture used for sleeping and resting was conducted in apartments from both groups at 8 and 16 wk. Bed bugs observed during visual inspection of apartments were left undisturbed. If total bed bug counts from interceptors and/or visual inspection exceeded 20, the apartment was discontinued from the experiment according to the IRB protocol, and property management was notified so the apartment could be scheduled for treatment. Apartments that were discontinued from the study were included in data analysis until the time they were discontinued.

Statistical Analysis

Bed bug count data were log-transformed prior to analysis of variance to compare differences among treatments. Non-parametric analyses were conducted on bed bug count data that could not fit normal distribution after transformation. Kruskal-Wallis test was used to compare the bed bug counts among treatment groups at 12 wk for Experiment I and at 8 wk for Experiment II. Data after these observation periods were not analyzed because apartments with bed counts ≥ 20 were discontinued from the experiments. Kruskal-Wallis test was also used to compare bed bug counts between apartments whose residents were aware or who were unaware of the presence of bed bugs in their apartments at the time of the initial inspection. Wilcoxon signed rank test was used to compare the mean bed bug count per trap at and away from sleeping and resting areas. All analyses were performed using SAS software version 9.3 (SAS Institute 2011).

Results

Experiment I. Trap Catch in Untreated and Treated Apartments with Low-level Infestations

The mean number of bed bugs based upon 14 d trap catch at 0 wk was similar in the three groups ($\chi^2 = 0.82$; df = 2; $P = 0.66$) (Table 4.1). Nymphs were trapped in 17-

22% of the apartments. Adult females were present in at least 78% of the apartments and adult males in ≤ 30% of the apartments in each group.

Bed bug counts per interceptor were similar in traps located at or away from host sleeping and resting areas in Group I (S = 54.5; $P = 0.10$), while more bed bugs were captured in traps located away from host sleeping and resting areas than those at sleeping and resting areas in Group II (S = -78.5; $P = 0.003$) and Group III (S = -73.5; $P = 0.01$) (Table 4.2). There was also a much higher percentage of apartments with bed bugs trapped away from sleeping and resting areas in Groups II and III apartments compared to Group I (Table 4.2).

Table 4.1. Summary of apartments used in Experiment I at 0 wk.

Treatment group	# of apts.	Mean bed bug count ± SEM	# of apts. with adult bed bugs trapped			# (%) apts. with nymphs trapped
			Female only	Male only	Female and male	
Never treated	23	2.7 ± 0.5	12	1	6	4 (17)
Recently treated with no additional treatment	23	2.4 ± 0.4	15	1	4	5 (22)
Recently treated with continued treatment	21	2.2 ± 0.4	14	0	4	4 (19)

Table 4.2. Trap count distribution within apartment and bed bug detection rates at 0 wk.

Treatment group	Average bed bug count by trap[1] (mean # traps per area) ± SEM		Average % of total trap catch		% of apts. with bed bugs trapped	
	At sleeping and resting areas[2]	Away from sleeping and resting areas	At sleeping and resting areas	Away from sleeping and resting areas	At sleeping and resting areas	Away from sleeping and resting areas
I. Never treated	0.19 ± 0.04 (10.1 ± 0.8)	0.07 ± 0.01 (17.7 ± 0.2)	44	56	61	78
II. Recently treated with no additional treatment	0.06 ± 0.02 (11.8 ± 0.7)	0.11 ± 0.02 (17.5 ± 0.2)	17	83	39	100
III. Recently treated with continued treatment	0.05 ± 0.02 (10.1 ± 0.6)	0.10 ± 0.02 (17.5 ± 0.2)	17	83	33	90

[1] Average bed bug count is the total number of bed bugs captured divided by the number of traps present in the area.

[2] Sleeping and resting areas refer to beds and upholstered furniture.

The bed bug counts at 12 wk declined to 0 in at least 71% of the apartments in all three groups. There were no significant differences in the mean bed bug counts among Groups I, II, and III ($\chi^2 = 5.07$; df = 2; $P = 0.08$) (Fig. 4.2).

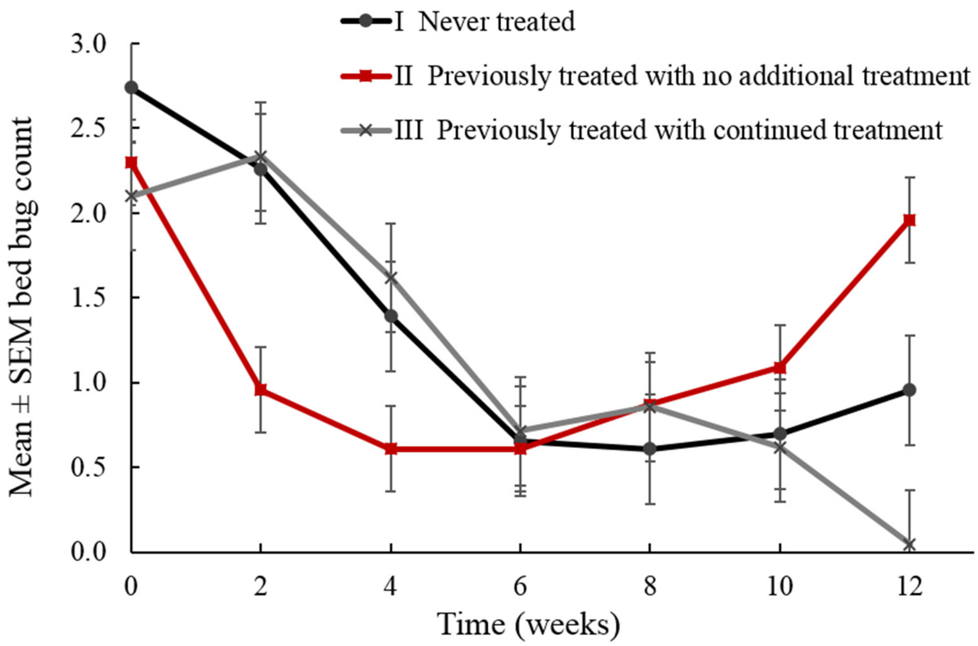

Figure 4.2. Mean ± SEM bed bug count in each apartment based upon total trap catch in each apartment during the first 12 wk in Experiment I. Data between 12 wk and 40 wk were not shown because two apartments were removed from the study.

Bed bug counts increased to 20 or more bed bugs in two apartments; one from Group I (20 bed bugs at 12 wk) and one from Group II (26 bed bugs at 20 wk). These two apartments were not inspected after the bed bug count reached ≥ 20 and they were considered still infested at 40 wk. At 22 wk, bed bugs had been eliminated in 96, 87, and 100% of the apartments in Groups I, II, and III, respectively (Fig. 4.3). At 40 wk, when the study was terminated, two apartments (Group II) still had bed bugs (counts were two and one, respectively).

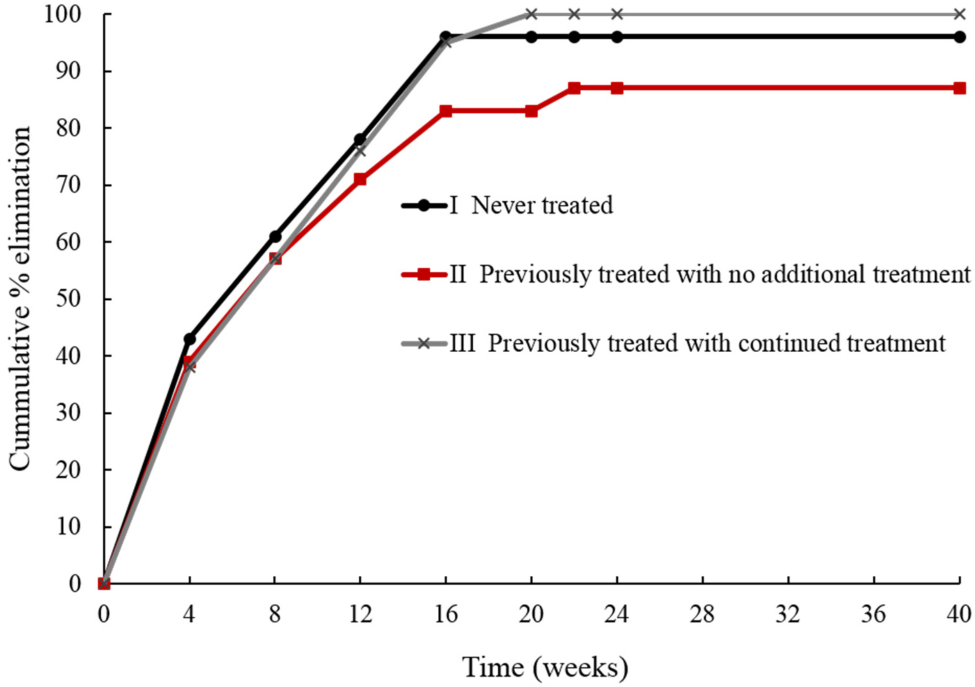

Figure 4.3. Cumulative percent elimination of infestations over time. Elimination is based upon four consecutive 14 d visits with zero trap catch and no live bed bugs observed during visual inspection.

Among all apartments, the mean number of visits to eliminate bed bugs and the mean number of visits that bed bugs were detected was 4.1 and 2.5, respectively. There were only two out of 65 apartments where bed bugs were observed during visual inspection following four consecutive visits without interceptor trap catch. Thus four consecutive visits without bed bug activity detected in interceptors were necessary to achieve 97% confidence of bed bug elimination in apartments (Fig. 4.4). Seven months after bed bugs had been eliminated new bed bug activity was reported by a resident in Group II. A total of eight bed bugs were detected on the sofa based on visual inspection and 14 d interceptor counts in this apartment. Two bed bugs were also detected in interceptors in one apartment from Group I, 12 mo after the infestation had been eliminated.

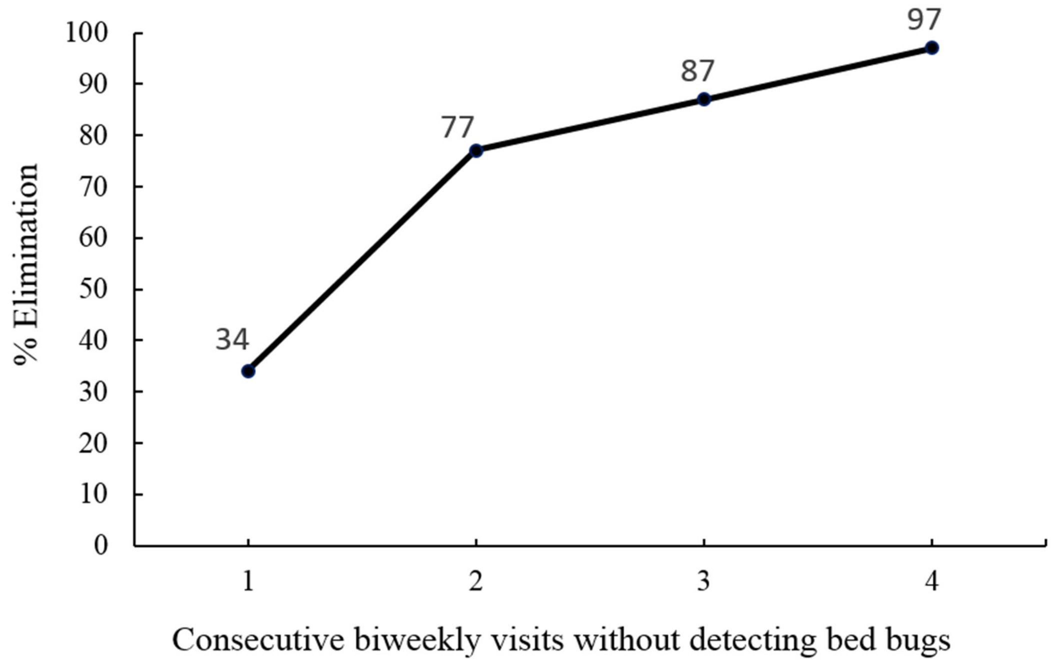

Figure 4.4. Relationship between the number of consecutive visits with zero bed bug counts in interceptor traps and elimination rate. A total of 65 infested apartments were included at 0 wk.

Experiment II. Impact of Interceptors on Low-level Bed Bug Infestations

The initial number of bed bugs and their sex distribution based upon trap catch and visual inspections are summarized in Table 4.3. The bed bug counts were similar between the two groups ($\chi^2 = 0.05$; df = 1; $P = 0.83$). Nymphs were trapped in 39 and 44% of the apartments in the treatment and control group, respectively. Adult females were captured in 56-71% of the apartments and adult males in no more than 22% of the apartments in each group (Table 4.3).

Table 4.3. Summary of apartments used in Experiment II at 0 wk.

Treatment group	# of apts.	# Residents aware of infestation[1]	Mean bed bug count ± SEM	# of apts. with adult bed bugs trapped			# (%) apts. with nymphs trapped
				Female only	Male only	Female and male	
Interceptors continuously present	18	12	2.8 ± 0.6	7	0	4	7 (39)
Interceptors present periodically[2]	18	11	2.4 ± 0.4	9	0	1	8 (44)

[1] Indicates the number of apartments in which the resident was aware of the infestation prior to being entered into the study.

[2] Interceptors were present between 6-8 wk and between 14-16 wk.

At 8 wk, the bed bug count was significantly lower ($\chi^2 = 9.11$; df = 1; $P = 0.003$) in the treatment group (5.2 ± 3.0) than in the control group (39.4 ± 21.0). The percentage of apartments with zero bed bugs at 8 wk was 61% and 11% in the treatment and control groups, respectively. We further analyzed the bed bug counts in apartments whose residents were aware and those who were unaware of the presence of bed bugs. At 0 wk, bed bug counts were similar in those who were initially aware and those who were unaware (treatment: $\chi^2 = 0.04$; df = 1; $P = 0.85$; control: $\chi^2 = 0.58$; df = 1; $P = 0.45$). At 8 wk, trap counts among apartments whose residents were aware were similar to those who were unaware in the treatment group ($\chi^2 = 2.4$; df = 1; $P = 0.12$), but were significantly higher than those who were unaware in the control group ($\chi^2 = 9.5$; df = 1; $P = 0.002$) (Table 4.4). Bed bug counts increased to ≥ 20 in eight apartments; two from the treatment group (37 and 43 bed bugs) and six from the control group with a mean ± SEM count of 108.2 ± 55.5. The eight apartments with bed bug counts over 20 were not inspected again and were considered still infested at 16 wk.

Table 4.4. Relationship between resident awareness of existing bed bugs and bed bug count based on interceptors and visual inspections.

Treatment group	Mean initial bed bug count ± SEM		Mean 8 wk bed bug count ± SEM	
	Aware[1]	Unaware	Aware	Unaware
Interceptors continuously present	3.1 ± 0.8 (n = 12)	2.2 ± 0.5 (n = 6)	7.8 ± 4.4 (n = 12)	0.2 ± 0.2 (n = 6)
Interceptors present periodically	2.8 ± 0.6 (n = 11)	1.7 ± 0.4 (n = 7)	63.3 ± 32.9 (n = 11)	1.9 ± 0.7 (n = 7)

[1] The resident's awareness is based upon resident interview prior to our detection of bed bugs in their apartment and historical pest control records.

At 16 wk, two more apartments had bed bug counts greater than 20 (both in the control group). All l0 apartments with bed bug counts that exceeded 20 bed bugs during the study period were in apartments whose residents were aware of the bed bug activity at the time we first detected them. Eleven (61%) apartments had zero bed bug counts in the treatment group. Among these, 9 had zero counts from 8 wk through 16 wk suggesting that bed bugs were eliminated in these apartments. In comparison, two (11%) apartments in the control group had zero counts. One of them also had zero bed bugs at 8 wk, suggesting that bed bugs may have been eliminated in at least one apartment.

Discussion

This study provides important information regarding the effects of various interventions on low-level bed bug populations. We found that many of the small populations of bed bugs were eliminated without any professional treatment and only a small percentage escalated in number over a period of 4-10 mo. The presence of the traps throughout the apartments represented a mass trapping approach and contributed to the decline of bed bugs in low-level infestations. These findings suggest that low-level infestations can be eliminated without insecticide applications and highlights the importance of early detection, and a threshold-based approach to bed bug management, by which the treatment protocol is based upon population size.

Previous studies have shown interceptors to be more effective than trained bed bug sniffing dogs (Cooper et al. 2014) or visual inspection (Wang et al. 2010, 2011; Cooper et al. 2014, 2015a) for detecting bed bugs present in low numbers. We used interceptors to identify 103 apartments (total number of apartments in Experiments I and II) with low-level bed bug activity. Of these, residents from 80 of the apartments were unaware that they had bed bugs. These results clearly demonstrate the effectiveness of pitfall traps as detection devices of bed bugs present in small numbers.

Using simulation models, Pereira et al. (2013) predicted rapid population growth starting with a single male and female bed bug. Under their worst scenario, when food was only made available once per week for 5 min at a time, populations increase up to 300 individuals in 15 wk with more rapid population growth rates predicted with increasing availability to food, as would be expected in an occupied residence. Our results suggest small populations rarely achieve their population growth potential under field conditions and that the introduction of a small number of bed bugs into previously un-infested apartments often fail to develop into high numbers, even when left untreated. Evidence of this can be seen in our first experiment among apartments in Group I. This group consisted of apartments that had no prior history of bed bug activity during the previous two years and whose residents were unaware of the bed bugs in their apartments prior to our detection. Since we initially trapped 10 or fewer bed bugs in each of these apartments, it is reasonable to assume that these populations likely represented recent introductions. Bed bugs were eliminated (based upon trap catch and visual inspection) in 22 out of 23 of these apartments within 22 wk without any treatment intervention. In our 2nd experiment, bed bug counts remained below 20 in a majority of the apartments with newly identified infestations, regardless of whether or not interceptors were continuously present. These results support the assertion that residential infestations detected early, can be eliminated with relative ease (Pinto et al. 2007, Wang and Cooper 2011, Vaidyanathan and Feldlaufer 2013).

The use of monitoring traps as a control method for urban pests has been limited to stored product pests, where pheromone traps have been used in mass trapping and mate disruption programs (Cox 2002, Phillips and Throne 2010). Schal and Hamilton (1990) pointed out that mass trapping does not appear to be a viable option for the control of cockroaches and that the lack of efficient trapping methods for cockroaches is probably the most significant single factor contributing to a heavy reliance on scheduled applications of insecticides. Wang et al. (2009) was the first to suggest that interceptors under the legs of beds and furniture may contribute to the reduction of bed bugs in infested apartments. The results of our first experiment demonstrate that most low-level populations of bed bugs are eventually eliminated even without treatment. One possibility is that interceptors placed throughout apartment remove bed bugs faster than they reproduce, contributing to the elimination of bed bugs present in small numbers. This was confirmed in our second experiment. Significant differences were observed in the dynamics of bed bug populations in apartments in the treatment group, which had interceptors continuously present for 16 wk, compared to those in the control group, which only had interceptors present periodically to obtain counts at 8 and 16 wk. The initial number of bed bugs present in apartments in Experiment II are likely to have been higher than in the first experiment due to differences in the number of interceptors placed per apartment for the initial detection of bed bugs, which may explain the lower elimination rates observed in the second experiment. In Experiment I interceptors were placed at and away from sleeping and resting areas, while in Experiment II they were only placed at sleeping and resting areas. Cooper et al. (2014, 2015b) demonstrated that bed bugs are often trapped in interceptors away from sleeping and resting areas, even in apartments with low bed bug counts. Thus because apartments in Experiment II did not have any traps away from sleeping and resting areas, the actual number of bed bugs at the start may be underestimated.

An Allee effect is a feature that exists in low density populations that limits population growth, such as failure to locate a mate when population size is small (Boukal and Berec 2009, Fauvergue 2012, Fauvergue et al. 2012). It is possible that such an effect, also contributed to the low population growth observed in this study. The host finding range of bed bugs is typically not more than 3 m (Marx 1955, Anderson et al. 2009, Singh et al. 2012). It has been suggested by Cooper et al. (2015b) that bed bugs that are more than a few meters from their host may become “lost” due to their inability to locate hosts. This could explain why bed bugs are commonly trapped in interceptors away from host feeding sites which may result in a decreased likelihood to locate a mate. At the onset of our first experiment, 56% of bed bugs in apartments that had never been treated were captured away from host sleeping and resting areas and up to 83% in apartments with infestations that had been treated. The differences in distribution between previously treated and untreated apartments could also be due to mortality of bed bugs at beds and furniture from treatment, as well as movement of bed bugs away due to application of insecticides. Romero et al. (2009) suggested that use of pyrethroids may present a potential problem for the spread of bed bugs. It is possible that pyrethroids used in the treatment of apartments in Groups II and III, along with other control practices may have facilitated the increased capture of bed bugs in interceptors away from the sleeping and resting areas and contributed to the persistence of bed bugs in

Group II compared to Group I. Whether movement away from the host affects host and mate finding warrants further investigation.

Dispersal of bed bugs from infested apartments to neighboring apartments has been implicated as a contributing factor in the spread of bed bugs within housing communities (Doggett and Russell 2008, Wang et al. 2010, Booth et al. 2012, Cooper et al. 2015a). Using mark-release-recapture, Cooper et al. (2015b) demonstrated active dispersal from five of six infested apartments to 42% of their neighboring apartments within 30 d. Moreover, the majority of actively dispersing adults captured in neighboring apartments were females. We also found females to be the more prevalent adult stage during our initial detection of bed bugs in the 103 apartments with low-level activity, regardless of whether the infestation was new or approaching elimination. It has been suggested that adult females are the primary dispersal stage in bed bugs (Pfiester et al. 2009, How and Lee 2010, Cooper et al. 2015b). This could explain why females are the dominant adult stage present in low-level bed bug populations. Dispersal of adult females would enable them to expand the infestation to other sleeping areas within the same living unit or neighboring units, as well as escaping control efforts targeted at host sleeping areas. However, the prevalence of adult females in our study could also be the result of trap bias for adult females compared to nymphs (both young and old) and adult males (Cooper et al. 2015b). For this reason we are unable to conclude that adult female bed bugs are the primary disperser. Booth et al. (2012) and Saenz et al. (2013) suggested that low genetic diversity among bed bug populations within the same apartment building indicates that most populations are founded by genetically related individuals and suggesting that a single female could give rise to an infestation. Based upon our results it seems likely that an introduction of a single female, or even a few bed bugs, may not readily become established. Instead, repeated introductions may be required.

We found bed bugs were more likely to remain low in number in apartments where residents were unaware of the presence of bed bugs compared to those were aware of the presence of bed bugs. Of the 25 residents who knew about the bed bugs in their apartments, 23 indicated they were self-treating their apartments, prior to the experiment, with one or more over-the-counter products, while none of the residents that were unaware were self-treating. In spite of the self-treatment of apartments in the "aware" group, these apartments had significantly higher bed bug counts than the "unaware" group at 8 wk in the control group. We speculate that infestations in apartments whose residents were initially unaware of the activity are likely to be new introductions that have not yet become established, while those in apartments that had received treatments and whose residents were aware may be established infestations with persistent low-level activity.

Bed bugs can be more difficult to detect towards the terminal end of a treatment effort than when first introduced. The likelihood of detecting bed bugs in traps placed at sleeping and resting areas versus away from sleeping and resting areas was similar in apartments that had not been treated (Group I). However among the 44 apartments that were treated (Groups II and III), 95% were detected in interceptors away from sleeping and resting areas and only 36% were detected in interceptors located at sleeping and resting areas. These results are similar to another study, where 47 of 67 apartments with bed bug activity were detected in interceptors located in areas such as kitchens, bathrooms, hallways and hall closets but not through visual inspection or interceptors at beds or upholstered furniture (Cooper et al. 2014). Since most of the bed bugs are found in less predictable areas away from sleeping and resting areas following treatment, placement of traps away from beds and upholstered furniture significantly increases the likelihood of detecting bed bugs in treated apartments.

A single service visit without detecting live bed bugs is commonly used by the pest control industry as an indication that bed bugs are no longer present. Once bed bugs are no longer found the treatment program is typically terminated. However, even with interceptors placed throughout the apartment, bed bugs were not detected in all apartments during every visit. Premature termination of treatment can result in chronic infestations and lead to the continued spread of bed bugs within communities (Wang and Cooper 2011). Based upon our results, four consecutive 14 d interval visits without activity provides at least 97% confidence that bed bugs have been eliminated. Bed bugs were only detected in two apartments, one at 7 mo post-elimination and the other at 12 month post-elimination, demonstrating the robust nature of our elimination protocol.

The results of our study have important implications that should be considered in the development of bed bug management programs in multi-unit housing communities, particularly those at risk for high infestation rates. These include: 1) installing pitfall-style traps both at and away from host sleeping and resting areas significantly improves detection following treatments; 2) mass trapping can effectively suppress low-level infestations; and 3) more than one service visit without detection of bed bugs should be used as a criterion for determining bed bug elimination.

Conclusion

New infestations that are small in number often fail to become established in occupied apartments, while small populations remaining from previously established infestations are more persistent and likely to escalate in number. Low-level populations are easily eradicated through placement of a large number of traps throughout apartments, reinforcing the importance of early detection. There are drawbacks of using mass trapping as the sole method of control because it takes more visits to eliminate infestations than if combined with other methods (i.e. encasement, steam, vacuum, or pesticide). Also, mass trapping alone may not be acceptable if occupants are being negatively affected by bed bugs (experiencing bite symptoms). In spite of these drawbacks, our results demonstrate that mass trapping has a significant impact on low-level bed bug populations. We recommend incorporating mass trapping into bed bug management programs to reduce the need for pesticide applications as well as to confirm elimination.

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CHAPTER FIVE

Evaluation of a model community-wide bed bug management program in affordable housing

Abstract

Low income apartment communities in the U.S. are suffering from disproportionally high bed bug, *Cimex lectularius* L., infestations due to lack of effective monitoring and treatment. Studies examining the effectiveness of integrated pest management (IPM) for the control of bed bugs in affordable housing have been limited to small subsets of bed bug infested apartments, rather than at the apartment community-level. We developed, implemented, and evaluated a complex-wide IPM program for bed bugs in an affordable housing community. Proactive inspections and biweekly treatments using a combination of nonchemical and chemical methods until bed bugs were not detected for three biweekly monitoring visits were key elements of the IPM program. A total of 55 bed bug infested apartments were identified during the initial inspection. Property management was unaware of 71% of these infestations. Over the next 12 mo, 14 additional infested apartments were identified. The IPM program resulted in a 98% reduction in bed bug counts among treated apartments and reduced infestation rates from 15% to 2.2% after 12 mo. Adopting a complex-wide bed bug IPM program, incorporating proactive monitoring, and biweekly treatments of infested apartments utilizing nonchemical and chemical methods can successfully reduce infestation rates to very low levels.

Introduction

The public in the U.S. is currently experiencing a resurgence of bed bug, *Cimex lectularius* L., infestations. The impacts associated with bed bug infestations can be physical, medical, mental, and economic in nature (Hwang et al. 2005, Goddard et al. 2009, Susser et al. 2012) all of which can be exacerbated in underserved communities (Rossi and Jennings 2010, Eddy and Jones 2011, Aultman 2013). In a 2012, a survey of 16 New Jersey housing authorities revealed that up to 40% of the units were infested (Wang C, unpublished data). In another 2012 survey, 65% of 26 affordable housing communities in Virginia reported having bed bug activity, 6.4% of the apartments had been treated for bed bug infestations with several communities suffering infestation rates between 8-19% (Wong et al. 2013). Moreover, individuals living in homes with bed bug activity often become victims of social injustice, being refused access to health care and other public services (Aultman 2013). In an effort to address bed bug infestations, residents often take matters into their own hands which can result in the misuse of pesticides with potentially harmful or dangerous consequences (CDC 2011, Doggett et al. 2012, Jones and Bryant 2012). Improperly applied pesticides increase the risk of negative health effects among residents, especially vulnerable populations like children and the elderly. Pesticide misapplication also increases selection pressure on bed bugs, potentially promoting the development of resistance. Bed bugs are particularly difficult to eliminate in low-income communities (Wang et al. 2009) where the necessary financial resources and knowledge to cope with the rapidly expanding bed bug infestations are often lacking (Rossi and Jennings 2010, Aultman 2013, Wang et al. 2010). Eradication efforts often require numerous service visits from a pest management professional and involves the use of a variety of chemical and nonchemical control measures, along with the selective treatment or disposal of infested furniture and other personal belongings (Wang et al. 2009, 2012, 2013). In spite of the challenging nature of bed bug management, affordable housing communities often hire pest management vendors based on the lowest bid for service (Singh et al. 2013b) and only schedule treatment when residents complain. Unfortunately, residents often fail to report bed bug infestations for a variety of reasons: they are unaware of the infestation, they are ashamed or embarrassed, they do not want to be bothered with invasive pest control procedures, or they fear negative repercussions by property management (Rossi and Jennings 2010, Eddy and Jones 2011, Wang et al. 2010). Failure to report infestations early on can result in established infestations that may spread to other apartments (Booth et al. 2012, Saenz et al. 2012) and are more difficult and costly to control (Ralph et al. 2013).

Affordable housing communities for elderly and disabled residents are especially at risk for high infestation and re-infestation rates (Wong et al. 2013). Ralph et al. (2013), found the elderly to be the demographic least likely to self-report infestations. An extensive study examining dispersal of bed bugs in a high rise housing community occupied by elderly and disabled residents found that 45% of the apartments were bed bug infested, and 53% of the apartments adjacent to infested apartments were also infested (Wang et al. 2010). In spite of the high infestation rates observed in their study, over 50% of the residents with infestations were unaware they had bed bugs in their apartments. Thus, relying on a reactionary bed bug management approach in multi-family housing communities, promotes development of severe/chronic infestations that can spread to other apartments and increase the cumulative costs of control.

Integrated Pest Management (IPM) originated as an agricultural concept and has been defined as a pest management system that utilizes all sustainable techniques in a compatible manner to reduce pest populations and maintain them at levels below those causing economic injury (EIL) (Smith and Reynolds 1965). Unlike agriculture where the primary objectives of IPM are to reduce costs and maximize gains, the primary objective of IPM for pests of the urban environment is the reduction of aesthetically displeasing pests that may also create public health hazards (Schal and Hamilton 1990). Often, acceptable pest thresholds are not based upon EIL, but rather, what a client is willing to tolerate and vary from one client to next. This differs for pests such as cockroaches, rodents or bed bugs that have public health implications, and for which the acceptable threshold is often zero. Most commonly, IPM methods include education, monitoring, and the implementation of nonchemical and chemical strategies. Wang and Cooper (2011) suggested that an IPM approach is necessary at the apartment community level to achieve effective eradication of existing bed bug infestations. IPM methods could also reduce the spread of bed bugs and decrease the cost of control by identifying new bed bug introductions in the early stages (Wang and Cooper 2011). In spite of the fact that IPM is widely recommended for the control of bed bugs in multifamily housing communities (Wong et al. 2013, Wang et al. 2009, Pinto et al. 2007), no studies examining the effectiveness of IPM at the apartment community level have been conducted. Instead, studies have focused on small subsets of apartments within infested communities (Wang et al. 2009, Wang et al. 2012, 2013; Singh et al. 2013b). These studies resulted in up to 97.6% reduction of bed bug populations, but never eliminated more than 67% of the treated infestations. Failure to eliminate bed bugs from infested apartments may result in chronic infestations that can endanger the financial stability of the property, health of residents, and further serve as a source of new infestations (Singh et al. 2013b). Wong et al. (2013) concluded that because bed bug infestations have public health, financial, and social justice implications, housing authorities must adopt more effective bed bug detection and control strategies.

Starting in 2012, we designed and implemented a model IPM program for the control of bed bugs in an affordable housing community for elderly and disabled residents in Jersey City, NJ. The program included education of property management staff and residents about bed bugs and their control. Inspections of apartments were conducted at the onset of the program to identify unreported infestations, as well as at 6 and 12 mo to evaluate the effectiveness of the program, and to identify other unreported infestations. Apartments with bed bugs were treated using an integrated management strategy that relied primarily on nonchemical measures with limited use of pesticides, applied in a targeted fashion. During the second half of the study we implemented a threshold-based approach in an effort to further reduce pesticide usage. Our primary objectives were 1) to reduce the apartment complex-wide infestation rate by at least 70% within 12 mo, and 2) to reduce the amount of pesticide usage over the course of the study. We evaluated the program's effectiveness by measuring reductions in the number of infested apartments, reductions in bed bug counts within infested apartments, and reductions in the amount of pesticides used over the course of the one year study.

Materials and Methods

Study site

The study was conducted at Jersey City Housing Authority located in New Jersey. The housing community consisted of four high-rise apartment buildings (A, B, C, and D) and a total of 358 apartments, of which 288 were one-bedroom, 54 studio, and 16 two-bedroom apartments. During the study period, 92-98% of the apartments were occupied. The residents were low income elderly (> 62 yr old) or disabled people. Among them, approximately 75% were African Americans, 20% were Hispanics, and 5% were of other ethnic groups. Based upon historical pest control records provided to us by property management, the first bed bug infestation in the community was reported in 2007. The number of known infested apartments rose from one apartment in 2007 to 32 reported apartments in 2008. Between 2008 and 2011, 118 apartments with bed bug infestations were treated, of which 46 apartments experienced repeat bed bug activity caused either by reintroduction of bed bugs or control failure. The infestations were treated by an in-house staff member licensed in the application of pesticides by the New Jersey Department of Environmental Protection. The in-house pest control staff used a variety of measures to control bed bugs including the use of mattress encasements, physical removal through vacuuming of bugs, application of steam, and the application of several pesticides including liquid residual, aerosol, and dust formulations. There was no consistency in the materials or methods used to treat one infestation to the next. Likewise, there was no protocol for follow-up services or when to stop treating infested units; both were left to the discretion of the in-house pest control technician. Treatment efforts were typically terminated when residents indicated that they were pleased with the results of the treatment effort.

Education of the apartment community

Educational seminar and resident survey. At the onset of the project, 11 management/staff members attended the bed bug training. Five of them were management level employees and the other six were general staff with varying roles such as maintenance, electricians, plumbers, painters, etc. The educational program was conducted in a classroom setting in a community room at the apartment complex. Education consisted of a one hour PowerPoint presentation projected onto a large (1.5 x 1.5 m) movie screen at the front of the room. Each of the attendees received a bed bug awareness poster, two bed bug fact sheets (each in English and Spanish), and a copy of the United States Department of Housing and Urban Development guidelines for multifamily housing (all items can be found at http://njaes.rutgers.edu/bedbug/?building-managers). In addition a short (7 min) video on bed bug IPM (http://njaes.rutgers.edu/bedbug/?videos#IPM) was projected onto the movie screen. Refreshments (food and beverages) were provided to attendees.

Resident training was held immediately following the staff training and was carried out in community rooms located in buildings A and B, and a shared community room for residents of buildings C and D. Notices announcing the training topic and date of the training were distributed to all residents one week prior to the training. The notice also advised residents that refreshments (food and beverage) would be provided during the training session. Residents were shown a 30 min PowerPoint presentation designed specifically for residents in multifamily housing (http://njaes.rutgers.edu/bedbug/?residents). Residents were also provided with two different bed bug fact sheets (in English and Spanish); however, the PowerPoint and video were only presented in English. Subjects discussed during both staff and resident training included the resurgence of bed bugs, basic biology, behavior, identification, prevention, and control as well as the roles and responsibilities for each audience. Key messages delivered during the educational session included: 1) Bed bugs do not discriminate - anyone get bed bugs regardless of cleanliness or social status; 2) If you suspect bed bugs it is important to report the problem to property management immediately; 3) If you have or suspect bed bugs it is not necessary to throw your bed away, most of the time beds can be saved; 4) Don't apply pesticides on your own, this may spread the infestation and can be potentially harmful to your health, leave pesticide applications to professionals who know how to treat the problem correctly; and 5) You can help eliminate bed bugs by frequently laundering bed linens and by eliminating clutter under, on, and immediately adjacent to beds and upholstered furniture.

At the beginning of the meeting, residents filled out a brief survey. Questions included: 1) are you aware of bed bug activity in your apartment at the present time; 2) have you ever experienced bed bug activity in your apartment; and 3) if, you previously have a bed bug infestation in your apartment, did you apply insecticides to control the bed bugs?

Distribution of bed bug fact sheets and resident interviews during initial inspection and 12 mo inspection of apartments. Apartments were inspected for bed bugs at the beginning of the study (initial inspection) and at 6 and 12 mo. Each of these inspections consisted of two visits 14 d apart. Residents were provided with bed bug fact sheets (3 pages) (in English and Spanish) during the first visit of the initial, 6 mo, and 12 mo inspections. A verbal interview was conducted with residents that were home during the second visit (14 d later) of the initial and 12 mo inspections. At the completion of every interview, resident's responses were discussed with the resident, to explain which answers were correct, incorrect, or partially correct. This was done in an effort to further educate the residents. Interview questions that were evaluated during both the initial and 12 mo inspections included: 1) do you believe bed bug infestations are caused by people who do not clean well; and 2) if a bed becomes infested with bed bugs is it necessary to discard the bed or are there methods to save it? Questions that were evaluated during the 12 mo interview only, include: 1) have you ever experienced a bed bug infestation and if so, how long ago; 2) If you experienced an infestation, did you develop bite symptoms; 3) if your apartment became infested with bed bugs, would you be very concerned, somewhat concerned, or not concerned; 4) if your apartment became infested with bed bugs, would you report the infestation to property management; and 5) how do you believe bed bugs are introduced into apartments (neighboring apartments, visitors, public places, 2nd hand items, bed wetting, other)? "Bed wetting" was included in the choices for question #5 because many residents mentioned bed wetting as a cause of infestation during our initial inspections.

Proactive procedure for new residents

A resident "move-in" procedure was implemented in an effort to discover new bed bug introductions in association with the arrival of new residents. When signing a new lease, the new resident was provided bed bug education materials by property management. Within one month after move-in, the Jersey City Housing Authority

(JCHA) pest control technician visited the new resident's apartment and inspected beds and upholstered furniture for bed bugs. If no bed bugs were observed, interceptors were installed under the legs of beds and upholstered furniture, and checked 14 d later.

Initial inspection of apartments

At the onset of the study, apartments in all four apartment buildings A, B, C, and D were visited and residents that were home were asked if they were aware of bed bug activity in their apartment at the present time or within the past 12 mo. Regardless of the resident's response to this question, an average of 10 Climbup® interceptors (Susan McKnight, Inc., Memphis, TN, USA), referred to hereafter as interceptors, were installed under the legs of beds and upholstered furniture in every unit in building A. Alternatively, in buildings B, C, and D, interceptors were only installed in apartments of residents who indicated; 1) that they were aware of, or suspected bed activity; or 2) their apartment had been treated for bed bugs by the housing authority within the past 12 mo. In total 53 of the 202 apartments in these three buildings were monitored. The rationale for this was to save cost, because of very low infestation rates in these three buildings based upon historical pest management records. In the previous 12 mo, a total of 15 out of 202 units were treated in buildings B, C, and D combined compared to 32 infestations in building A (156 units). All apartments with interceptors were inspected 14 d later by 2-3 Rutgers University researchers, and a visual inspection of the beds and upholstered furniture was conducted if no bed bugs were observed in the interceptors.

Treatment of infestations

All treatments were performed by the licensed in-house pest control technician employed by JCHA. Rutgers University researchers assisted in the treatment of the first three infested apartments to allow the in-house pest control technician to become familiar with the treatment protocol. A second housing authority staff assisted in lifting heavy beds and furniture. Two months after the onset of the study, the 2nd staff member was no longer available due to a labor shortage. All treatment data were transferred to Rutgers researchers and analyzed for effectiveness of the IPM program. The protocols for the initial treatment and follow-up services were as follows:

Initial treatment. Bed linens and any clothing on floors were bagged and residents were provided with laundering instructions and tokens to offset the expense of laundering. Mattresses and box springs were encased (AllerZip®; Protect-A-Bed®, Northbrook, IL, USA). An Omega Green Supreme IPM HEPA vacuum (Atrix International, Burnsville, MN, USA) was used to remove visible bugs and a Steamax steamer (AmeriVap® Systems, Dawsonville, GA, USA) was used to apply hot steam to upholstered furniture, bed frames, headboards, footboards, and furniture within 90 cm of beds. Pesticide applications during initial treatments were limited to two low impact products, MotherEarth® D (100% diatomaceous earth; Whitmire Micro-Gen Research Laboratories, St. Louis, MO, USA) and a pro-insecticide Phantom® aerosol (0.5% chlorfenapyr; BASF, Research Triangle Park, NC, USA). MotherEarth D was applied using a bulb duster along baseboards and outlets and switch plates, located behind beds and upholstered furniture, and extending 90 cm to either side of sleeping and resting areas. The same dust was also applied inside upholstered furniture by poking the duster through the dust cover on the underside of the furniture. Phantom® aerosol was applied around the perimeter on the underside of furniture as well as around the legs and beneath the fabric skirt if one existed. Phantom aerosol was also used to treat other areas where bed bug activity was observed. Interceptors were installed under legs of bed frames and upholstered furniture. Additional interceptors were placed at the corners of living room and bedroom, as well as one in each closet, the bathroom, and kitchen.

At 6 mo into the study, a "threshold-based" treatment protocol was introduced for the initial treatment in apartments with newly identified bed bug activity. Control measures in apartments identified with 5 or fewer bed bugs, based on interceptors and visual inspections, were limited to nonchemical methods only that included physical removal of bugs, encasement of the mattress and box spring, and installation of interceptors as described above. If bed bug counts greater than five were observed during one of the follow-up service visits other measures, including the use of pesticides were made available. The threshold of 5 or fewer bugs is conservative, and was based upon the results of a previous study in which we eliminated bed bugs from 77% (30 of 39) of apartments with initial bed bug counts of ten or fewer bed bugs, using nothing more than encasements and installation of a similar number of interceptors as this study (Cooper et al. 2015c).

Follow-up service visits. During each service visit, residents were asked if they were aware of any new activity (seeing bed bugs or being bitten). All interceptors were inspected and either maintained (cleaned and talc powder re-applied) or replaced depending upon their conditions. A visual inspection of bed and upholstered furniture was also conducted during each service visit. When the total number of bed bugs from interceptors and visual inspection combined was five or fewer, the visible bugs were physically removed. If more than five bugs were observed, live bugs were removed and the area of activity was treated using one or more of the following: 1) steam; 2) vacuum;

3) MotherEarth D; 4) Phantom aerosol; or 5) Transport® GHP liquid residual spray (0.05% acetamiprid and 0.06% bifenthrin; FMC Corporation, Philadelphia, PA, USA) applied with a one gallon B & G sprayer (B&G Equipment Co., Jackson, GA, USA). If bed bugs were still found after five months from initial treatment, more aggressive measures such as discarding infested items or heating of infested items in a portable heat chamber (Thermal Strike® Expedition Bed Bug Heat Treatment, Fort Collins, CO, USA) were implemented at the technician's discretion.

Follow-up service visits continued on a biweekly basis until three criteria were met over three consecutive 14 d intervals: 1) No bed bugs captured in any of the interceptors, 2) no bed bug activity observed during visual inspection of beds and upholstered furniture, and 3) no new reports of bed bug activity or bite symptoms by the resident. Once these criteria were met, follow-up service visits were terminated and the infestation considered resolved. Three consecutive visits of no observed activity was selected based upon previous unpublished results showing that the chance of finding bed bugs again was <10% (Cooper RA, unpublished data).

Pesticide use. The amount of pesticide used in each apartment was recorded by measuring the weight of the dust bulb and aerosol can immediately before and after each treatment using a Salter balance (model #1015; Salters Housewares, Oakbrook, IL, USA). The amount of liquid residual applied was estimated by comparing the volume of the solution in the B&G sprayer before and after each application.

Evaluation of the effectiveness of the IPM program

Inspections of all 358 apartments in four buildings were conducted at 6 and 12 mo. These inspections served two purposes; 1) to detect unreported bed bug infestations, and

2) to evaluate the program effectiveness. To detect unreported infestations, interceptors were installed under the legs of beds and upholstered furniture in all occupied apartments that were not currently being treated in all four buildings and checked 14 d later for bed bugs. A visual inspection was conducted if no bed bugs were found in the interceptors and any one of the following conditions was met: 1) the unit was treated for bed bugs during the 6 mo prior, 2) the resident believed bed bugs were present in the unit, and 3) the resident moved in after the initial inspection. Visual inspections were also conducted in units adjacent to apartments that had 50 or more bed bugs and were treated within the previous 6 mo.

The effectiveness of the IPM program was measured at the conclusion of the 6 and 12 mo inspections using the following parameters: 1) changes in the number of infested apartments, 2) changes in mean bed bug count, and 3) changes in the amount of pesticides used.

Cost of the IPM program

The cost of the IPM implementation was measured by calculating the labor and material cost for inspections, treatments, and pesticide usage. During inspections, the time spent in apartments as well as time between units, waiting for residents, unlocking doors, and other down time encountered were recorded. Labor cost was $50 per hour based on JCHA estimate. Costs for equipment were not included in the cost calculation because the housing authority already owned all of the equipment necessary (duster, vacuum, steamer, and compressed air sprayer) and these tools are typically owned by those providing bed bug management services. The cost of education was limited to those associated with the printing of materials and time for staff to attend training. Costs for the delivery of education was not included in the cost calculation because training is available free of charge through extension service or pest management vendors. Additionally, all of the educational materials used in this study are available to the public at http://njaes.rutgers.edu/bedbug. PowerPoint presentations are available with full narrative text and are also available in a video format. The 7 min IPM video and the video of the resident PowerPoint are available in English and Spanish. The pesticide use was compared with that used in other published bed bug management studies in low-income communities.

Statistical analysis

Only responses from English speaking residents were used for the analysis of questions asked during the educational seminar and the resident interviews during the initial and 12 mo inspection. A Chi-square test (SAS/STAT Users Guide, 2011) was used to compare responses of residents to two questions asked during both the initial and 12 mo interviews. A Chi-square test was also used for analyzing if level of concern about bed bugs is related to whether they had an infestation within the past 12 mo. Regression analysis was conducted to evaluate the relationship between the number of treatment visits required to eliminate a bed bug infestation and the logarithmic transformed initial bed bug count. The relationship between the total amount of pesticide usage and the logarithmic transformed initial bed bug count was also analyzed using regression analysis. One outlier with a very large initial bed bug count (1,413 bed bugs) was excluded from the analysis. The number of service visits to eliminate infestations in the apartments identified during the initial inspection and those identified after the initial inspection was compared using analysis of variance.

Results

Education of the apartment community

Survey during the seminar. Residents from 167 (47%) apartments attended the bed bug education meeting and filled out the survey. Fifty two percent of the respondents (n = 121) indicated that they either had a current infestation (16) or previously had an infestation (47) in their apartment. Of the 63 residents that had experienced bed bugs in their apartment, 56% indicated that they applied pesticides on their own to treat the problem.

Interviews during home inspections. Table 5.1 lists questions and answers asked during initial and 12 mo interview, or 12 mo interview only. The percentage of residents that believed bed bugs are caused by a lack of cleanliness remained similar at the initial and the 12 mo interview ($\chi^2 = 0.01$; DF = 1; P = 0.91). However, the number of residents that said infested beds must be discarded decreased significantly during the 12 mo interview compared to the initial interview ($\chi^2 = 13.9$; df = 1; $P = 0.0002$). Among residents that said they had experienced a bed bug infestation during their lifetime, 76% of the infestations had occurred within last 10 years. There was no relationship between the level of concern expressed by residents about getting bed bugs and their previous infestation history within the past 10 years (n = 133) ($\chi^2 = 3.69$; df = 2; $P = 0.16$). Additionally, infestation history had no impact upon whether residents would report the infestation to property management.

Table 5.1. Resident interview questions and answers during home inspections[1]

Question	n	Percentage answered yes
1. Are bed bug infestations caused by lack of cleanliness?	59	Initial inspection – 56% 12 mo inspection – 42%
2. Is it necessary to discard a bed that had become infested with bed bugs?	60	Initial inspection – 77% 12 mo inspection – 42%
3. Do you have current bed bug infestation or previously have an infestation?	145	53%
4. Did you experience bite symptoms? Only those had bed bugs in the past 10 years were included.	59	40%
5. If your apartment becomes infested with bed bugs, how concerned would you be?	133	Very concerned - 68% Somewhat concerned - 15% No concern - 17%
6. Would you report the infestation to the management office if your apartment became infested?	142	Have bed bugs – 87% Do not have bed bugs – 87%

[1] The results for question #3-6 are from the 12 mo interview.

Regarding the source of infestations, residents were familiar with the following ways that bed bugs can be introduced: 2nd hand items such as used furniture (97%); visitors to the apartment (91%); neighboring apartments (80%); and from public places (79%). Surprisingly, 38% of the residents believed bed wetting was one of the ways bed bug infestations occurred.

Initial inspection results and treatment of apartments

Initial inspection. A total of 209 out of 358 apartments were inspected. All of the apartments in building A (156) were inspected, along with 31 of 130, 14 of 36, and 8 of 36, in buildings B, C and D, respectively. Inspections were completed by 2 researchers over a 7 wk period (27 June 2012 – 14 August 2012). A total of 2,077 interceptors were installed under the legs of beds and furniture (mean = 10 interceptors per apartment). The mean time required for installation of interceptors was 4.5 min per apartment. During the 14 d follow-up inspection, interceptors were inspected in all 209 apartments, and visual inspections were conducted in 81 of the apartments. The mean time to inspect interceptors and to conduct visual inspections was 6.6 and 16 min per apartment, respectively (not including down time between apartment inspections).

Fifty-five apartments with bed bug activity were identified. JCHA was unaware of 71% of the infestations. Interceptors detected 95% of the infestations and 5% were detected by visual inspections after interceptors failed to reveal the presence of bed bugs. The number of apartments identified with bed bug activity in buildings A, B, C, and D were 39, 4, 8, and 4, respectively. Among the 55 apartments with bed bug activity, 25 had < 10 bugs, 20 had 10-50 bugs, 6 had 51-100 bugs, and 4 had >100 bugs. The mean (min, max) bed bug count in interceptors was 66.4 (1, 1,413) based upon a 14 d trapping period.

Initial treatment of apartments with bed bug activity. The 55 apartments with bed bug activity were treated by the in-house pest control technician between 25 June 2012 and 21 August 2012. The mean time required to provide the initial treatment was 102 min per apartment. The mean amount of chemical applied per apartment was 12.1 g of Mother Earth and 62.6 g of Phantom aerosol. Initially, each apartment was serviced by two people, the in-house pest management technician, and a helper to assist with lifting and moving of heavy furniture, bagging of clothing, organizing equipment, etc. However, after just two weeks the helper was eliminated due to a shortage in staff, leaving 23 apartments to be treated by the in-house technician, without any assistance.

Inspection results and evaluation of IPM program at the conclusion of six and twelve month inspections

Six month inspection. A total of 304 out of 358 apartments were inspected. Among the 54 apartments not inspected, 30 were vacant, 6 were occupied by residents that either refused access (4) or had private locks (2), and 18 were being treated for bed bugs. The inspections took 2 researchers 7 wk to complete (4 January 2013 – 21 February 2013). A total of 2,912 interceptors were installed under the legs of beds and furniture (mean = 10 interceptors per apartment). The mean time required for installation of interceptors was 2.1 min per apartment. During the 14 d follow-up inspection, interceptors were inspected in all 304 apartments, and visual inspections were conducted in 54 of the apartments. The mean time to inspect interceptors and to conduct visual inspections was 3.7 and 15.6 min, respectively (not including down time between apartment inspections).

Seven apartments with bed bug activity were identified during the 6 mo inspection. Six of the infestations identified were detected by interceptors and one through visual inspection after interceptors failed to detect any bed bugs. Four of the apartments with activity were in building A, two in building B, and one in building C. The mean (min, max) bed bug count among the seven apartments was 2.4 (1, 8). Two of the seven apartments with bed bug activity were not monitored during the initial inspection. None of the residents in the seven apartments were aware that bed bugs were present in their units.

Six month evaluation of IPM program. Prior to the 6 mo inspection, two apartments with bed bug activity were reported by residents, one in building C and the other in D. Neither of these apartments had been inspected during the initial inspection at the onset of the study and both had fewer than 5 bugs based upon interceptor trap catch and visual inspection.

By the conclusion of the 6 mo inspection, a total of 64 apartments had been identified with activity (Table 5.2). Among them, 62 were treated. Bed bugs were eliminated from 52 apartments, leaving 10 treated apartments with bed bug activity. Among the treated apartments still with bed bugs, five were identified during the initial inspection and the other 5 were identified after the initial inspection. The mean bed bug count among treated apartments was reduced by 96% by the conclusion of the 6 mo inspection. The infestation rate was reduced from 15% to 2.8%. Bed bugs were not detected in any of the apartments where infestations had already been eliminated, thus at 6 mo the recurring infestation rate was zero.

Table 5.2. Summary of inspection and treatment results.

Inspection	# of newly identified apts. with bed bug activity	# of apts. treated	# of infestations eliminated	# of apts. with recurring infestations	# of apts. remaining with bed bug activity
Initial	55	NA[1]	NA	NA	NA
End of initial inspection –end of 6 mo inspection	9[2]	62	52	0	10[3]
End of 6 mo inspection–end of 12 mo inspection	5[4]	4	9	2	8[5]
Overall	**69**	**66**	**61**	**2**	**8**

[1] Not applicable.

[2] Two of these apartments were reported between the end of the initial inspection and the start of the six month inspection.

[3] Five of these apartments were from the initial 55 apartments treated and 5 were from apartments identified between the initial inspection and conclusion of the six month inspection.

[4] Two of these apartments were reported between the end of the six month inspection and the start of the 12 mo inspection

[5] Three of these apartments were from the initial 55 apartments (one was never eliminated and 2 were recurring), 2 apartments were identified between the end of the six month inspection and conclusion of the twelve month inspection, and 3 apartments were identified during the 12 mo inspection. The 3 new infestations and 2 recurring infestations were not treated until after the study was concluded.

A total of 12,262 g of finished product (Phantom aerosol – 8,849 g, Transport GHP – 2,528 g, and Mother Earth – 885 g) was used to treat 62 infested apartments through the end of the six month inspection. A mean of 198 g of pesticide was used in the treatment of 62 apartments.

Twelve month inspection. A total of 325 out of 358 apartments were inspected. Among the 33 apartments not inspected, 27 were vacant, 3 were occupied by residents that had private locks and could not be accessed, and 3 were still being treated for bed bugs. The inspections took two researchers 6 wk to complete (1 July 2013 – 10 August 2013). A total of 3,346 interceptors were installed under the legs of beds and furniture (mean = 10 interceptors per apartment). The mean time required for installation of interceptors was 2.1 min per apartment. During the 14 d follow-up inspection, interceptors were inspected in all 325 apartments, and visual inspections were conducted in 51 of the apartments. The mean time to inspect interceptors and to conduct visual inspections was 2.9 and 16.3 min per apartment, respectively (not including down time between apartment inspections).

Five apartments with bed bug activity were identified during the 12 mo inspection. Three of them were new infestations and two were apartments with recurring bed bug activity that had been treated during the first six weeks of the study. All five infestations were detected by interceptors. Three of the apartments were in building A and the other two apartments were in building C. Four apartments, had counts between 1-3 bed bugs, however, one unit had approximately 500 bed bugs in interceptors and 4,000 bed bugs based on visual inspection. No bed bugs had been detected in this unit during the previous inspection at 6 mo, however, during the 12 mo inspection bugs were visually observed crawling all over bed sheets. Residents in all 5 of the apartments, including the heavily infested one, indicated that they were not aware of the bed bug activity.

Twelve month evaluation of IPM program. Prior to the 12 mo inspection, two new apartments with bed bug activity were reported by homemakers providing in-home care to the resident. Both of these apartments were in building A. One of the apartments had a bed bug count of 7 bugs and the other had 14 based upon interceptor trap catch and visual inspection.

Over the course of the study, there were 69 unique apartments infested with bed bugs (55 at onset, 2 between initial and 6 mo inspections, 7 during 6 mo inspection, 2 between 6 and 12 mo inspections, and 3 during 12 mo inspection). Among these 69 apartments, 71% were identified through proactive inspections. Ninety-four percent of the infestations identified during proactive inspections were detected by interceptors. Four units were identified by residents or home-health aides and 16 units were already known to the housing authority. During the study, 15 new residents moved into the housing community. No bed bug activity was identified during the inspection of these 15 apartments by the in-house pest control technician, as part of the "new move-in" protocol. Recurring bed bug activity was detected during the 12 mo inspection in two apartments treated during the first 6 wk of the study, bringing the total number of bed bug occurrences to 71. Of the 71 occurrences, 66 were treated and five were scheduled to be treated after the conclusion of the study.

The infestation rate at the conclusion of the initial, 6 mo and 12 mo inspections was 15%, 2.8%, and 2.2%, respectively. Overall, bed bugs were eliminated in 92% (61 out of 66) of the treated apartments (Table 5.2), and the mean bed bug count was reduced by 98%. Among the 5 apartments still with activity, two were identified just prior to the 12 mo inspection, one was identified during the initial inspection and had received 22 service visits but still had 9 bed bugs at 12 mo and the other two were apartments with recurring activity, each with only 1 bed bug found at 12 mo inspection.

The mean number of service visits required to eliminate infestations identified during the initial inspection was significantly greater (8.2 visits) compared to those identified after the initial inspection (2.7 visits) (F = 8.8; df = 1; $P < 0.004$) (Fig. 5.1).

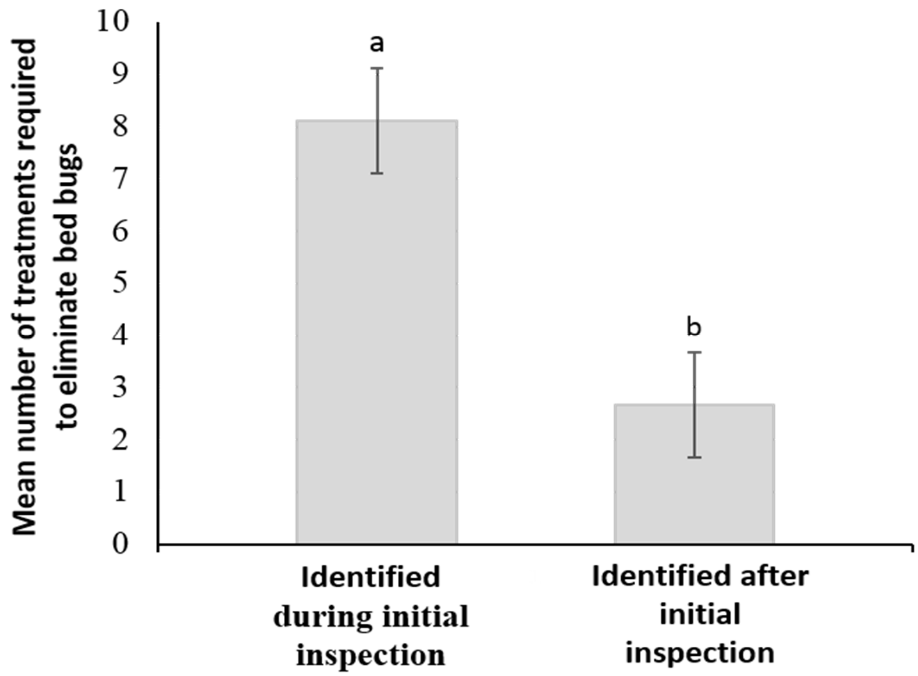

Figure 5.1. Mean number of treatments to eliminate infestations identified during initial inspection (n = 52) compared to apartments identified after the initial inspection (n = 9). Bars with different letters are significantly different (*P* < 0.05; ANOVA).

There was a significant correlation between the initial bed bug counts and the number of treatment visits required to eliminate an infestation (F = 47.3; df = 1, 59; $P < 0.0001$; $R^2 = 0.45$) (Fig. 5.2).

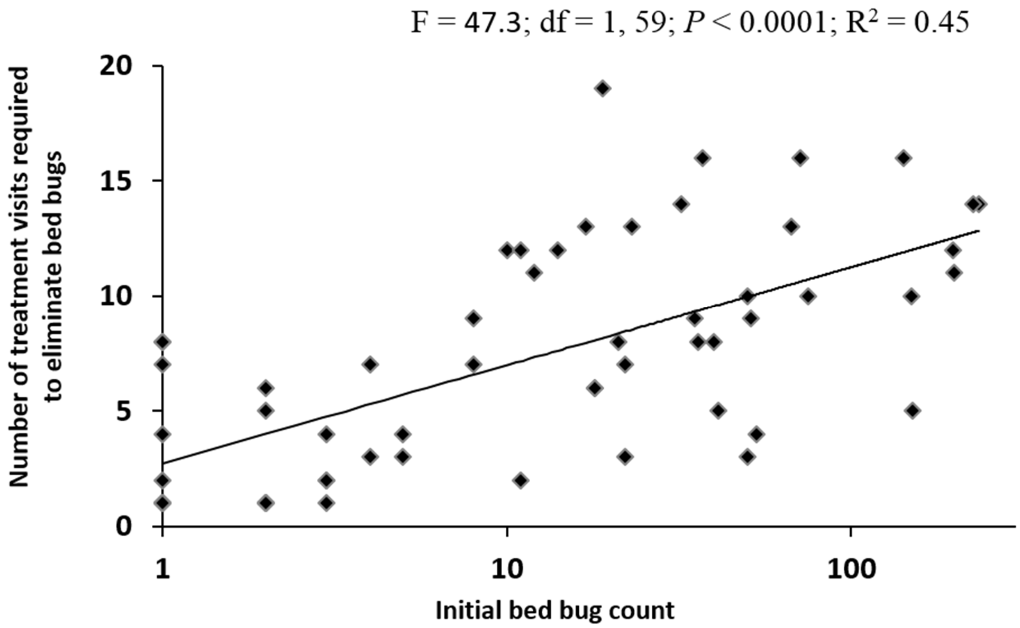

Figure 5.2. Correlation between bed bug counts and number of treatment visits required for eliminating an infestation.

Among the treated apartments, 60% of the residents told the in-house pest control technician that they experienced no bite symptoms and 62% were not aware that they had an infestation in their apartment. Prior to bugs having been eliminated from their apartment, 76% of the residents indicated to the in-house pest control technician that they believed their apartment was no longer infested, even though bed bugs were still detected during biweekly inspections.

A total of 13,248 g of finished product (Phantom aerosol – 9,537 g, Transport GHP – 2,809 g, and Mother Earth – 902 g) was applied over 12 mo to treat 66 infested apartments (Table 5.3). A mean quantity of 201 g of finished product was used per apartment. Four of the six infested apartments identified during the 6 mo inspection with fewer than five bed bugs were serviced using the threshold-based treatment protocol, the other two apartments, were accidentally treated with chemical during the initial service.

Table 5.3. Total pesticide used for treating 66 apartments.

Time Period	Finished Pesticide Product Applied[1]			
	Phantom (g)	Transport GHP (g)	Mother Earth (g)	Total applied (g)
Start – end of 6 mo inspection	8,849	2,528	885	**12,262**
End of 6 mo insp. – end of 12 mo inspection	688	281	17	**986**
12 mo total	**9,537**	**2,809**	**902**	**13,248**

[1]The respective formulation types for Phantom, Transport GHP, and Mother Earth are aerosol, liquid, and dust, respectively.

Regression analysis revealed a significant positive correlation between the amount of pesticide usage and the initial bed bug count (F = 35.6; df = 1, 64; $P < 0.0001$; $R^2 = 0.36$) (Fig. 5.3). The quarterly pesticide usage is shown in Fig. 5.4. As the number of active infestations decreased from the 1st quarter to the 4th quarter, pesticide usage decreased by 94%.

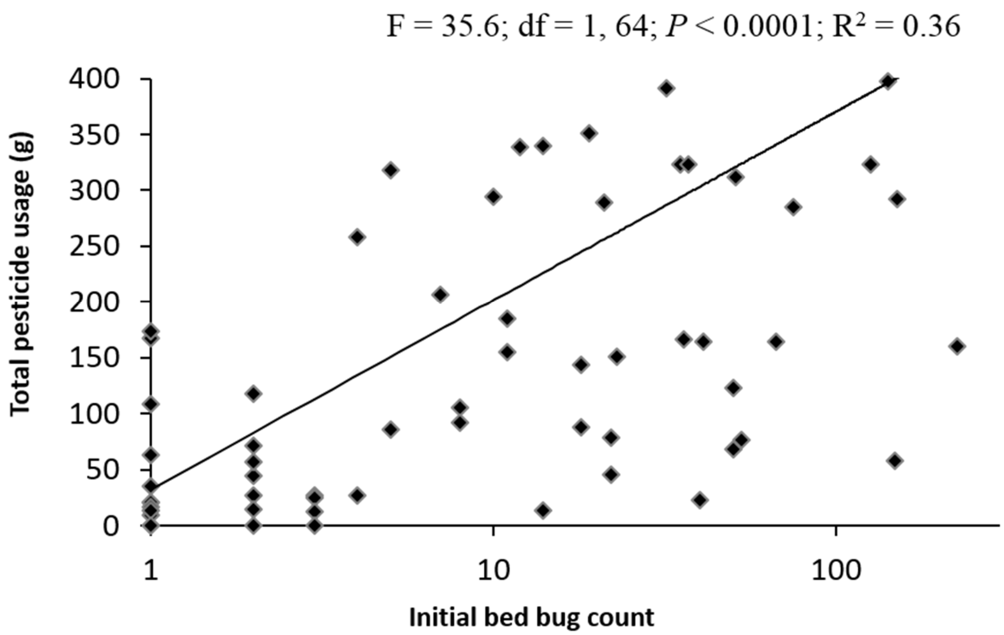

Figure 5.3. Correlation between bed bug counts and amount of pesticides used per apartment.

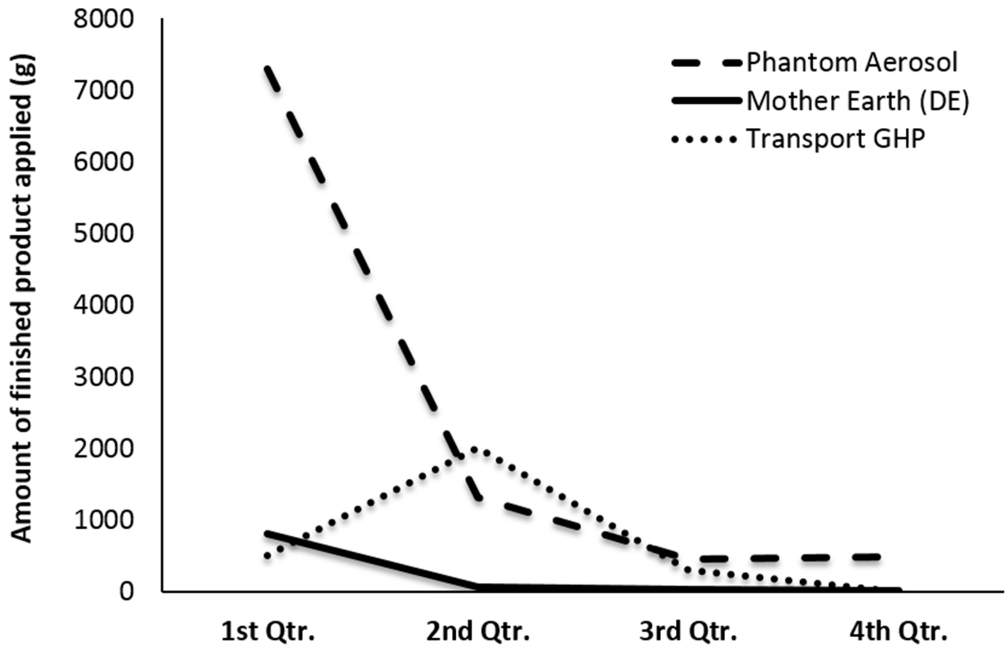

Figure 5.4. Quarterly insecticide usage for treating apartments with bed bug activity.

Cost of the IPM program

Costs for the IPM program are summarized in Table 5.4. Interceptors were purchased directly from the manufacturer at a cost of \$2.00 per interceptor. A total of 9,897 interceptors were installed during inspections and treatment of apartments. The mean number of interceptors per apartment for inspections was 10 with an additional 11 added in apartments being treated for bed bugs, as part of the treatment protocol. A total of 350 man hours were invested for the community-wide inspections. The labor spent for 0, 6, and 12 mo inspections was 129, 112, and 109 h, respectively. Nonproductive "down time" between apartment inspections accounted for 61% of the labor (213 h). A labor rate of \$50 was used based upon the salary, including benefits, for the in-house technician. The total costs for community-wide education, inspection, and treatment were \$868,

$34,600 and $30,068, respectively. The average cost for treatment of 66 apartments (labor and materials) was $456 per apartment.

Table 5.4. Cost of the IPM program.

		Materials				Total
	Labor	**Intercep-tors**	**Encase-ments**	**Pesticide**	**Other**	
Education	$600[1]				$268[2]	**$868**
Inspection	$17,500	$17,100	$0	$0	$0	**$34,600**
Treatment	$21,010	$2,694	$5,411	$319	$634[3]	**$30,068**
Subtotal	**$38,510**	**$19,794**	**$5,411**	**$320**	**$1,608**	
	Grand total					**$65,536**

[1]wages paid to staff to attend educational training session.

[2]includes cost for refreshments, paper, toner, and copier maintenance to print educational materials and meeting announcements.

[3]includes cost of laundry tokens and heavy duty 30 gallon plastic bags.

Discussion

Our study is the first documented success of complex-wide IPM in an affordable housing community. The purpose of the study was not to evaluate specific treatment methods but rather to examine the effectiveness of an overall approach for the complex-wide management of bed bugs. The high level of control achieved is largely attributed to several practices: 1) a baseline inspection to identify unreported infestations; 2) a protocol for when to stop follow-up treatments and inspections; 3) periodic inspections for the continued early detection of unreported infestations; and 4) using a combination of nonchemical methods (installing encasements, applying steam etc.) and chemical methods to treat existing infestations. The use of interceptors, rather than relying on visual inspections, also contributed greatly to the results achieved.

Education of staff and residents, regarding biology, behavior, and what actions to take is an important component of any IPM effort in multi-family housing. However, in our study the educational effort produced mixed results. Between the initial and 12 mo interview, education had little impact on residents' perception that bed bugs are caused by a lack of cleanliness. However education was effective in changing the opinion of residents regarding what to do with beds that are infested with bed bugs. This was at least partially due to the fact that the housing staff installed mattress encasements in all infested apartments and therefore eliminated the need to discard the infested beds.

Over the course of the study, we noticed that a significant number of people believed bed bug infestations were caused by bed wetting behavior. It is possible that this belief is limited to the demographic present in this study as all of the residents explained that they learned this as small children from their parents. Consideration should be given to include this topic in future educational material to dispel this misconception.

Bed bug management strategies are often reactionary in nature, with treatment of infestations occurring as they are reported to property management (Wang et al. 2009, Singh et al. 2013b). This approach is problematic because residents often fail to recognize and report infestations which promotes the spread of bed bugs, can result in high infestation rates in multi-occupancy, and provides property management with a false understanding of the number of infestations that actually exist. At the onset of our study, management was unaware of 71% of the infestations. Through verbal interviews during service visits, we learned that residents in 62% of the apartments with bed bugs were unaware that bed bugs were present. Most of these residents (60%) were not experiencing bite symptoms, a phenomenon common among elderly individuals (Potter et al. 2010). These results are similar to those reported by Wang et al. (2010) where only 50% of the elderly residents interviewed in an affordable housing community were aware of bed bug activity in their apartments. Our results clearly illustrate that relying on the reporting of bed bug infestation by residents is unreliable, promotes increased infestation size, and furthers the spread of bed bugs to other apartments.

Periodic inspections at 6 and 12 mo were important for detecting unreported infestations. Following the initial inspection, a total of 16 apartments with bed bug activity were identified. Only 25% of these were reported to management by residents or home-health aides, the rest were the result of proactive inspections at 6 and 12 mo. Moreover, periodic inspections facilitated early detection of infestations. Saenz et al. (2012) concluded that early detection and mitigation of bed bug infestations is critical because infestations are generally started by only a few individuals. Our results support this conclusion and demonstrate that early detection allows for early treatment, requires fewer service visits and less pesticide to eliminate an infestation, and reduces spread of bugs compared to higher level infestations that are well established. Surprisingly, during the 12 mo inspection an apartment with over 4,000 bed bugs was identified. This apartment had no prior bed bug history and no activity detected during the initial or 6 mo inspection. The apartment was more cluttered during the 12 mo inspection than during the 6 mo inspection. Piles of papers, magazines and clothing were strewn about on the sofa and throughout the apartment. Further investigation revealed that the bugs in this heavily infested apartment were apparently introduced in heavily infested packages received from a relative less than 2 mo before the 12 mo inspection. It was also clear that the residents in the apartment had no intention of reporting the bed bug infestation to property management. Fortunately, as a result of the periodic inspections the problem was detected shortly after the bugs were introduced, subverting potential negative impacts on other apartments.

We demonstrated that a high level of bed bug population reduction is possible with an in-house pest management program in affordable housing where multiple obstacles to control exist. More importantly, we not only reduced bed bug numbers but also achieved a high elimination rate. Previous studies have achieved population reduction of > 90%, but had low elimination rates (Wang et al. 2009, 2010, 2013; Singh et al. 2013b, Moore and Miller 2009, Potter et al. 2006). By the end of the 12 mo study, we achieved 92% elimination among the treated infestations, reducing the community-wide infestation rate from 15% to 2.2%. Wang et al. (2010) suggested that a concentrated effort and greater financial input are very important in buildings with widespread infestations. Our results support this assertion. The high level of success achieved in our study was not realized without a great deal of persistence and vigilance. A mean number of 7 service visits were required in the 61 apartments where bugs were eliminated. Similar numbers of visits have been reported in studies by Potter et al. (2006, 2012),Wang et al. (2009, 2012, 2013), and Singh et al. (2013b), where up to 66% of the apartments continued to experience bed bug activity even after treatment for 12 wk or more. However, in our study a much higher elimination rate was achieved. Wang et al. (2013) pointed out that the time to eliminate an infestation can take a few months or more, depending on infestation level, complexity of the environment, cooperation from the building occupants, and thoroughness of the treatment procedures.

A variety of challenges contributed to the high number of service visits required to eliminate some of the infestations. During the first few months of the study, the in-house pest control technician was adjusting to the new treatment protocol and did not adhere to the biweekly follow-up service schedule. The lack of a 2^{nd} staff member to assist in some of the initial treatments and most of the follow-up service visits, also compromised the quality and speed of the services. Finally, some residents did not follow technician's instructions to reduce clutter and/or launder regularly, to assist the treatments. These factors contributed to the weak correlation between the initial bed bug count and 1) the number of service visits to eliminate the infestation, and 2) the quantity of pesticides applied. For example, three of the initial 55 apartments treated had relatively low initial bed bug counts of 17, 19 and 33 but required 13, 19, and > 22 service visits, respectively to eliminate infestations. The mean quantity of pesticide applied (658 g) in these three apartments was above the mean quantity (201 g) for the 66 apartments treated. All three of these apartments were very cluttered, with bed bugs dispersed amongst items away from beds and upholstered furniture. Two of the apartments also had very heavy furniture which was difficult for the technician to move without a helper.

Lack of resident cooperation is commonly cited as a cause for control failure even after months of repeated treatments (Wang et al. 2009, 2012, 2013; Singh et al. 2013b, Potter et al. 2006, 2012). To overcome inherent problems among low income seniors, residents in our study were not asked to carry out any preparations prior to treatment. Instead we took a more proactive stance. During each service visit the staff bagged linens along with other infested items that could be laundered (i.e. stuffed animals, pillows, clothing etc.) and residents that were not laundering their linens on a weekly basis were provided with tokens to encourage cooperation and offset the costs of laundering. Residents were also provided with heavy duty garbage bags to offset the expenses associated with de-cluttering. Occasionally it was necessary for the technician to assist residents, particularly those with disabilities, with the de-cluttering process and in a few cases a portable containerized heating box was employed to address items that could not be laundered or placed in a dryer. Over the course of the study resident cooperation improved. A possible explanation for this is that residents observed a new level of commitment from property management in the bed bug control since this IPM program was implemented.

Perhaps the most significant aspect of our IPM program was the implementation of an “elimination” protocol. Seventy-six percent of the residents in our study mistakenly believed their units to be free of bed bugs while bed bugs were still present. This finding supports the idea that bed bugs often go undetected when their numbers are low and that the decision, when to stop bed bug treatment, should not be based solely on resident satisfaction. Wang et al*.* (2009) reported similar results in another study, where following treatment of 16 apartments, none of residents complained about bed bug bites despite the fact that bed bugs were still detected by the authors in 50% of the apartments. In our study, we defined bed bug elimination as the absence of bed bugs based upon a combination of interceptor catch, visual inspection, and resident feedback for three consecutive visits. Moreover, interceptors were not only installed under legs of beds and furniture, but were also placed throughout the apartment based upon the findings of

Wang et al. (2010) and Wang and Cooper (2012), decreasing the likelihood of premature termination of the eradication effort. The protocol proved to be very effective and prevented premature termination of follow-up service visits. Of the 63 apartments where bed bug counts were reduced to zero, bed bugs re-appeared in 26 of them again after one or two more inspections. Thus the criterion of three consecutive visits without activity is important to prevent premature termination of the treatment. Following this criterion, only two out of the 63 apartments declared bed bug free, experienced bed bug activity following termination of service. Only one bed bug was detected in each of these two apartments and each had been removed from treatment for at least 6 mo, suggesting a re-introduction rather than control failure in the two units. The very low re-occurrence rate demonstrates the robust nature of the elimination criterion and treatment program implemented in this study.

The use of interceptors proved invaluable for identification of infestations, guiding treatments, evaluation of the treatment program, and ultimately the success of the IPM program (Wang et al. 2009, 2010). Ninety four percent of the apartments identified through proactive inspections were detected by interceptors placed under the legs of beds and upholstered furniture. Among the 286 visual inspections conducted in units where no bugs were detected by interceptors, only 4 additional infestations were found. Thus using interceptors is a reliable method for complex-wide bed bug detection. Moreover, it requires less expertise than conducting visual inspections. Efficiencies were gained in the complex-wide monitoring as we became more familiar with the residents and their apartments, and infestation rates dropped. The time required to install and inspect interceptors decreased by over half during the 6 mo inspection compared to the initial inspection. The average time to install and inspect interceptors during the 12 mo inspection was 2.1 and 2.9 min, respectively, compared to 4.5 and 6.6 min during the initial inspection. In spite of their simplicity and effectiveness, the value of interceptors for the detection, monitoring, and control of bed bugs is largely unrealized by pest management professionals and property managers of multi-family housing communities. In a survey of 251 pest management professionals, Potter et al. (2013a) reported that 99% conduct visual inspections to identify bed bugs while only 50% used interceptors in their detection programs. The results of our study demonstrate the effectiveness of interceptors for detecting bed bug activity and suggest that using interceptors is cost-effective for large scale inspections.

Placing interceptors away from the furniture played an important role in the control effort. It was not uncommon for bed bugs to be captured in interceptors away from sleeping and resting areas even though no bugs were observed at beds and upholstered furniture. This helped prevent premature termination of the follow-up program and provided information that influenced treatment decisions during follow-up service visits. For example, in several apartments, bed bugs captured in over 50% of the interceptors away from beds and furniture, prompted treatment of all baseboards with Transport GHP resulted in a rapid decrease in the widespread activity. In other apartments, the location of trapped bugs, led to increased additional inspection of closets, resulting in the location of bugs that may otherwise been missed. It has also been suggested that interceptors may contribute to the control of bed bugs by removing trapped bed bugs (Wang et al. 2010, 2013; Singh et al. 2013b).

Reducing pesticide use and exposure are key goals of an IPM program. However, the majority of the pest management industry continues to use pesticides as the primary tool for the control of bed bugs. In a survey of pest management professionals, 94% not only relied on pesticides, but also typically treated beds with them. Early detection through periodic inspections coupled with a low impact treatment protocol contributed to the very low pesticide usage. Our control strategy relied mostly on nonchemical measures and at no time was pesticide applied to beds. Instead, mattresses and box springs were encased and visible bed bugs were removed or destroyed using a vacuum, commercial steamer, or hand removal with forceps. When pesticides were used, applications were targeted mostly to areas where bed bug activity was observed. Additionally, control measures were limited to physical removal of visible bugs during follow-up service visits when bed bug counts were reduced to below five. Generalized treatment of baseboards throughout the apartment with liquid residuals was limited to just three of 66 apartments where bed bug bed bug activity was widespread based upon interceptor trap catch. An average of 201 g of finished product was applied to treat 66 apartments, which was ≥ 90% less compared to other reported field studies (Moore and Miller 2009, Potter et al. 2012). Also contributing to the reduction in pesticides applied was the threshold-based nonchemical protocol implemented at 6 mo for newly identified apartments with an initial count of 5 or fewer bugs. Bed bugs were eliminated from all 4 of the apartments where the nonchemical only protocol was applied, suggesting for very low level populations, elimination is possible without the use of pesticides. These results also provide evidence in support of the assertion made by Wang et al. (2009) that the use of interceptors is even more pronounced when bed bug numbers are low because they catch the few bed bugs present, reduce the risk of population build up, and reduce the need for pesticides.

The success of an IPM program is of little value if it is not economically viable and sustainable. The average annual cost for bed bug management at Berry Gardens during the two years prior to this study was approximately $57,215 per yr and failed to effectively manage the bed bug problem. In comparison, the total cost to implement our IPM program was $65,536 and yielded a dramatic reduction in the bed bug infestation rate. Proactive inspections accounted for 54% of the total costs but were integral to the success of our program. Potter et al. (2013a) questioned whether property managers could be convinced to pay for proactive inspections. Based upon our results, not implementing a proactive inspection is more costly in the long run in communities with high infestation rates. Following the initial year, the cost of the periodic inspections is reduced by approximately 33% because the initial inspection is no longer necessary. In addition, inspection costs can be further reduced by eliminating visual inspections and relying on interceptor trap catch for detection of bed bugs. Visual inspections accounted for approximately 11% ($3,800) of the inspection costs and only resulted in identification of 4 out of 71 infestations. Although not yet tested, we also believe the community-wide inspection cost can be further reduced by at least another 25% through restricting one of the two annual inspections to apartments with activity in the previous 6 mo. Treatment of 66 apartments accounted for 46% of the costs. The mean cost of treatment per infested unit in our study was $456. This is similar to the estimated treatment cost of $463-$482 and $445 per apartment reported by Wang et al. (2009) and Wong et al. (2013), respectively. While the cost of treatment is similar to other reported costs, a major difference is that bed populations were eliminated and not just reduced in the apartments treated in our study. Assuming an annual new infestation rate of 3-4%, total costs to maintain the IPM program in all four buildings are projected to be between \$21,045 and \$22,456 per yr (average of \$59– 63 per apartment per yr) and are likely to be much less because high level infestations should be rare, requiring less time and material to eliminate. For example, bed bug activity in six of 9 apartments identified between the 6 and 12 mo inspection were eliminated in a single service visit. Four of these infestations were eliminated without any pesticide application. It is also expected that the number of infestations will continue to decrease. We also believe that after two years of maintaining very low infestation rates (≤ 3%), the two community-wide inspections per year can be reduced to one community-wide inspection and a second inspection limited to apartments with bed bug activity during the previous 12 mo. A modified approach such as this would easily reduce inspection costs by two-thirds, bringing the annual cost to maintain the community-wide bed bug IPM program down to \$15,521 - \$16,785. Further field evaluation would need to be done to confirm if this modified inspection protocol is sufficient to maintain low infestation rates.

In conclusion, our bed bug IPM program provided a model that is both effective and economically practical for implementation in affordable housing communities suffering from chronic bed bug infestations. We also demonstrated the reporting of infestations by residents is unreliable. The protocol would not have been effective without the dedication of the in-house technician that implemented it. This point should not be overlooked, as many pest control contracts are based upon low bid and may lack the dedication and attention to detail necessary for a high level of success. Obstacles from lack of resident cooperation can be reduced through education of residents and increased assistance from the housing staff. Ongoing education and commitment of the housing staff will play an important role in the complete eradication of bed bugs.

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CONCLUSION

Application of findings in the management of bed bugs

Visual inspection of beds and upholstered furniture is unreliable for detection of bed bugs, particularly in apartments that have previously been treated. While trained canines are more attractive to consumers and useful in certain environments, pitfall style interceptors are more effective and economical for bed bug inspections. Placement of interceptors throughout the apartment, at and away, from sleeping and resting areas, increases the ability to detect bed bugs when their numbers are small. Three consecutive 14 day intervals without bed bugs detected in interceptors placed throughout apartments is necessary for a high degree of confidence (87%) that the bed bugs have been eliminated.

The mobility of bed bugs was found to be much greater than previously believed and has significant implications regarding how we monitor for bed bug activity, treat infestations, and manage the spread of bed bugs. Movement of bed bugs, particularly adult females, away from aggregations can promote the spread of bed bugs within apartments, and throughout multi-occupancy buildings. Control programs should be designed to take bed bug mobility into account when designing inspection and treatment protocols and treatments. Inspection and control efforts should be conducted in all areas of an infested dwelling (apt, home, hotel guest room etc.) and not limited to rooms where bed activity is believed to exist. It is recommended that living units neighboring a known infestation be inspected for bed bug activity, including units adjacent, above, below, and across the hall from the known infestation. This study also confirmed that bed bugs can survive long periods of time in the absence of a host. Based upon these findings infested dwellings that are vacated are at risk for continued infestation if reoccupied within five months post vacancy, particularly if the infestation was severe.

There are two circumstances when bed bugs are present in low numbers; 1) when a few bed bugs are first introduced through passive or active dispersal, and 2) at the terminal end of a treatment effort for an existing infestation. Low-level bed bug populations do not rapidly escalate in number. Mass trapping with interceptors in apartments with low-level populations plays a role in controlling the infestation. Bed bug populations can often be eliminated through mass-trapping alone.

A community-wide bed bug IPM approach to manage bed bugs is proven to be very successful. The infestation rate was reduced by 87% and the amount of pesticide applied was reduced by 92% during the second half of the study compared to the first six months. Most significantly, it is important to conduct proactive and periodic inspection of apartments to identify bed bug infestations that have not been reported to property management. Residents in over half (62%) of 71 apartments where bed bugs were identified, indicated they were unaware the bed bug activity existed. Failure of residence to recognize and report infestations can promote the continued spread of bed bugs within the community and lead to chronic infestation of the housing community. Additionally, infestations that become well established are more difficult to eliminate compared to those that are detected early on, when bed bug numbers are small. The treatment and follow-up protocol demonstrated that bed bugs could be eliminated in apartments without relying on pesticides and that the implementation of a rigorous elimination protocol (no bed bugs in interceptors or during visual inspection for three consecutive visits) prevents premature termination of the follow-up visits. Unfortunately, many affordable housing communities continue to employ a reactionary approach to bed bug management, only treating apartments after they have been reported to property management. This study provides a model for effective bed bug management in other housing communities.

Based upon the findings of my research, a number of recommendations can be made for the detection and management of bed bugs in multi-unit dwellings.

1. **Implement periodic bed bug inspections.**

   For buildings with bed bug infestations in multiple units within the past 12 months, community-wide bed bug inspections should be conducted once per year to identify new infestations that have not been reported by residents. A more targeted bed bug inspection should be conducted every six months for apartments that have been treated for bed bugs within the previous year. These apartments are at an increased risk for bed bug activity either due to reintroduction or because infestations were reduced to very low numbers but not eliminated.

2. **Employ a combination of at least two detection methods**

   Detection of bed bugs should not be limited to a single method. A combination of visual inspection plus interceptors at sleeping and resting areas is more effective than visual inspection or interceptors alone. Apartments where bed bugs are not detected during visual inspection, but have signs of activity or recent history should be monitored with interceptor traps at beds or both beds and upholstered furniture, for more accurate results.

3. **Treatment of bed bug infestations should not be based upon pesticides alone**

   A combination of nonchemical and chemical methods should be used to treat bed bug infestations. Encasing beds can immediately reduce bed bug numbers and increase the efficiency of follow up visits. Steam and vacuums are also very efficient methods for efficiently eliminating visible aggregations of bed bugs.

4. **Install additional interceptor traps away from sleeping and resting areas in infested apartments.**

   Placement of interceptor traps away from sleeping and resting areas serves multiple purposes including: evaluation of treatment effectiveness, reducing the number of bed bugs and intercepting bed bugs traveling in areas away from sleeping and resting areas. When infestations are reduced to a low-level, traps contribute to the elimination of the infestation. Recommendations for trap placements away from sleeping and resting areas include: one trap in each corner of the bedroom and living room, one trap in each closet, one trap in the hallway, bathroom, kitchen, and entry door.

5. **Conduct follow-up visits every two weeks until infestations are eliminated**

   Follow up visits should include an inspection of sleeping resting area and traps, with additional treatment based upon inspection findings. Follow up visits should continue until no bed bugs are found for at least 2 consecutive visits. Three consecutive visits will provide a higher (87%) level of confidence that infestations have been eliminated.

6. **Provide support for residents who are unwilling or unable to cooperate with essential requests for cooperation.**

   Requests for cooperation such as decluttering in areas of known infestation (beds, sofas, infested closets), laundering bed linens and infested clothing, and addressing other personal items that are infested (i.e. books, shoes, electronics etc.) are essential for the elimination of infestations. Providing assistance to residents by helping to declutter, assisting with laundering, and providing a method for disinfesting items that cannot be laundered (i.e. portable heat chamber, or bagging and freezing items for four days) will greatly reduce the elimination failures.

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LITERATURE CITED

Aak, A., B. A. Rukke, A. Soleng, and M. K. Rosnes. 2014. Questing activity in bed bug populations: Male and female responses to host signals. Physiol. Entomol. 39: 199–207.

Abdoul-Nasr, A. E., and M. A. S. Erakey. 1967. On the behaviour and sensory physiology of the bed bug: 1. Temperature reactions (Hemiptera: Cimicidae). Bull. Soc. Entomol. d'Egypte 51: 43–54.

Abdoul-Nasr, A. E., and M. A. S. Erakey. 1968a. Behaviour and sensory physiology of the bed bug, *Cimex lectularius* L., to some environmental factors: chemoreception (Hemiptera: Cimicidae). Bull Soc. Entomol. d'Egypte 52: 353–362.

Abdoul-Nasr, A. E., and M. A. S. Erakey. 1968b. The effect of contact and gravity reactions upon the bed bug, *Cimex lectularius* L. Bull Soc. Entomol. d'Egypte 52: 363–370.

Adelman, Z. N., K. A. Kilcullen, R. Koganemaru, M. A. E. Anderson, T. D. Anderson, D. M. Miller. 2011. Deep sequencing of pyrethroid-resistant bed bugs reveals multiple mechanisms of resistance within a single population, PLoS ONE 6: e26228. Doi: 10.1371/journal.pone.0026228.

Adelman, Z. N., D. M. Miller, and K. M. Myles. 2013. Bed bugs and infectious disease: a case for the arboviruses. PLoS Pathog. 9: e1003462. Doi: 10.1371/journal.ppat.1003462.

Aldrovandi, U. 1603. De Animalibus Insectis Bologna.

Anderson, J. F., F. J. Ferrandino, S. McKnight, J. Nolen, and J. Miller. 2009. A carbon dioxide, heat and chemical lure trap for the bed bug, *Cimex lectularius*. J. Med. Vet. Entomol. 23: 99–105.

Araujo, R. N., F. S. Costa, N. F. Gontijo, T. C. M. Gonçalves, M. H. Pereira. 2009. The feeding process of *Cimex lectularius* (Linnaeus 1758) and *Cimex hemipterus* (Fabricius 1803) on different bloodmeal sources J. Insect Physiol. 55: 1151–1157.

Aultman, J. E. 2012. Don't let the bedbugs bite: the Cimicidae debacle and the denial of healthcare and social justice. Med. Health Care and Philos. 16: 417–427.

Balvín O., P. Munclinger, L. Kratochvíl, and J Vilímová. 2012. Mitochondrial DNA and morphology show independent evolutionary histories of bedbug *Cimex lectularius* (Heteroptera: Cimicidae) on bats and humans. Parsitol. Res. 111: 457–469.

Barbarin, A. M., R. Gebhardtsbauer, and E. G. Rajotte. 2013. Evaluation of Blood Regimen on the Survival of *Cimex lectularius* L. Using Life Table Parameters. Insects 4: 273–286.

Bell, W. J. 1990. Searching behavior patterns in insects. Annu. Rev. Entomol. 35: 447–467.

Benoit, J. B., N. A. Del Grosso, J. A. Yoder, and D. L. Denlinger. 2007. Resistance to dehydration between bouts of blood feeding in the bed bug, *Cimex lectularius*, is enhanced by water conservation, aggregation, and quiescence. Am. J. Trop. Med. Hyg. 76: 987–993.

Benoit 2011. Stress tolerance of bed bugs: a review of factors that cause trauma to *Cimex lectularius* and *C. hemipterus*. Insects 2: 151–172.

Blow, J. A., M. J. Turell, A. L. Silverman, and E. D. Walker. 2001. Stercorarial shedding and transtadial transmission of hepatitis B virus by common bed bugs (Hemiptera: Cimicidae). J. Med. Entomol. 38: 694–700.

Boase, C. 2008. Bed bugs (Hemiptera: Cimicidae): an evidence-based analysis of the current situation. pp. 7–14. In: W. H. Robinson and D. Bajomi (eds), Proceedings of the 6th International Conference on Urban Pests, OOK-Press Kft., Hungary.

Booth, W., V. L. Saenz, R. G. Santangelo, C. W. Wang, C. Schal, and E. L. Vargo. 2012. Molecular markers reveal infestation dynamics of the bed bug (Hemiptera: Cimicidae) within apartment buildings. J. Med. Entomol. 49: 535–546.

Booth, W., O. Balvín, E. L. Vargo, J. Vilímová, and C. Schal. 2015. Host association drives genetic divergence in the bed bug, *Cimex lectularius*. Mol. Ecol. 24: 980–992. Doi: 10.1111/mec.13086.

Boukal, D. S., and L. Berec. 2009. Modelling mate-finding Allee effects and populations dynamics, with applications in pest control. Pop. Ecol. 51: 445–458.

Brooks, S. E., F. M. Oi, and P. G. Koehler. 2003. Ability of canine termite detectors to locate live termites and discriminate them from non-termite material. J. Econ. Entomol. 96: 1259–1266.

Browne, C., K. Stafford, and R. Fordham. 2006. The use of scent-detection dogs. Ir. Vet. J. 59: 97–102.

Busvine, J. R. 1976. Insects, hygiene and history. The Athlone Press. University of London. London, U.K.

Busvine, J. R. 1980. Insects and hygiene: the biology and control of insect pests of medical and domestic importance. 3rd ed. Chapman and Hall, London.

Cablik, M. E., and J. S. Heaton. 2006. Accuracy and reliability of dogs surveying for desert tortoise (Gopherus agassizili). Ecol. Appl. 16: 1926–1935.

Carayon, J. 1966. Traumatic insemination and paragenital system. pp. 81–166. In: R. L. Usinger (ed.), Monograph of Cimicidae (Hemiptera, Heteroptera), Entomological Society of America, College Park, MD.

Centers for Disease Control and Prevention. 2011. Acute illnesses associated with insecticides used to control bed bugs – seven states, 2003-2010. MMWR. 60: 1269–1274.

Cooper, R. 2007a. Are bed bug dogs up to snuff? Pest Control 75(1): 49–51.

Cooper, R. 2007b. Just encase. Pest Control 75(4): 64–66, 68, 70, 72–75.

Cooper, R. 2011. Bed bugs and kissing bugs. pp. 587–632. In: Hedges S. (ed.), Mallis' handbook of pest control, 10th edn. GIE Media Publications, Cleveland, OH.

Cooper, R., and H. Harlan. 2004. Bed bugs and kissing bugs. pp. 495–529. In: Hedges S. (ed.), Mallis' handbook of pest control, 9th edn. GIE Media Publications, Cleveland, OH.

Cooper, R., C. Wang, and N. Singh. 2014. Accuracy of trained canines for detecting bed bugs (Hemiptera: Cimicidae). J. Econ. Entomol. 107: 2171–2181.

Cooper, R. A., C. Wang, and N. Singh. 2015a. Evaluation of a model community-wide bed bug management program in affordable housing. Pest Manag. Sci. Doi: 10.1002/ps.3982.

Cooper, R. A., C. Wang, and N. Singh. 2015b. Mark-release-recapture reveals extensive movement of bed bugs (*Cimex lectularius* L.) within and between apartments. PLoS ONE 10: e013642. Doi: 10.1371/journal.pone.0136462.

Cooper, R., C. Wang, and N. Singh. 2015c. Effects of various interventions, including mass trapping with passive pitfall traps, on low-level bed bug populations in apartments. J. Econ. Entomol. 108: Doi: 10.1093/jee/tov337.

Cowan, F. 1865. Curious facts in the history of insects. J.B. Lippincott & Co. Philadelphia, PA.

Cox, P. D. 2002. Potential for using semiochemicals to protect stored products from insect infestation. J. Stored Prod. Res. 40: 1–25.

Davies, T. G. E., L. M. Field, and M. S. Williamson. 2012. The re-emergence of the bed bug as a nuisance pest: implications of resistance to the pyrethroid insecticides. J. Med. Vet. Entomol. 26: 241–254.

Delaunay, P., V. Blanc, P. Del Giudice, A.Levy-Bencheton, O. Chosidow, P. Marty, and P. Brouqui. 2011. Bedbugs and infectious diseases. Clin. Infect. Dis. 52: 200–210.

Doggett, S. L., and R. C. Russell. 2008. The resurgence of bed bugs, *Cimex* spp. (Hemiptera: Cimicidae) in Australia, pp. 407–25. In: W. H. Robinson and D. Bajomi (eds.). Proceedings of the 6th International Conference on Urban Pests, OOK-Press Kft., Hungary.

Doggett, S. L., M. J. Geary, and R. C. Russell. 2003. Has the tropical bed bug, *Cimex hemipterus* (Hemiptera: Cimicidae), invaded Australia? Environ. Health. 3: 80–82

Doggett, S. L., C. J. Orton, D. Lilly, and R. C. Russell. 2011. Bed bugs: the Australian response. Insects 2: 96–111.

Doggett, S. L., D. E. Dwyer, P. F. Peñas, and R. C. Russell. 2012. Bed bugs: clinical relevance and control options. Clin. Microbiol. Rev. 25: 164–185.

Duggan, J. M., E. J. Heske, R. L. Schooley, A. Hurt, and A. Whitelaw. 2011. Comparing detection dog and livetrapping surveys for a cryptic rodent. J. Wildl. Manag. 75: 1209–1217.

Eddy, C., and S. C. Jones. 2011. Bed bugs, public health, and social justice: part 1, a call to action. J. Environ. Health 73: 8–14.

Fauvergue, X. 2012. A review of mate-finding Allee effects in insects: from individual behavior to population management. Entomol. Exp. Appl. 146: 79–92.

Fauvergue, X., E. Vercken, T. Malausa, and R. A. Hufbauer. 2012. The biology of small, introduced populations, with special reference to biological control. Evol. Applic. 5: 424–443.

Furton, K. G., and L. J. Myers. 2001. The scientific foundation and efficacy of the use of canines as chemical detectors for explosives. Talanta 54: 487–500.

Furton, K., J. Greb, and H. Holness. 2010. The scientific working group on dog and orthogonal detector guidelines (SWGDOG). National Criminal Justice Reference Service, U.S. Department of Justice, Rockville, MD.

Garner, K. J., L. Busbee, P. Cornwell, J. Edmonds, K. Mullins, K. Rader, J. M. Johnston, and J. M. Williams. 2001. Duty cycle of the detector dog: a baseline study. Final Report. FAA Grant #97-G-020. Institute for Biological Detection Systems. Auburn University, Auburn, Alabama.

Gazit, I., A. Goldblatt, and J. Terkel. 2003. Explosives detection by sniffer dogs following strenuous physical activity. Appl. Anim. Behav. Sci. 81: 149–161.

Gazit, I., A. Goldblatt, and J. Terkel. 2005. The role of context specificity in learning: the effects of training context on explosives detection dogs. Anim. Cogn. 8: 143–150.

Getty, G. M., R. L. Taylor, and V. R. Lewis. 2008. Hot house. Pest Control Technol. 36: 96 –100.

Goddard, J., and R. deShazo. 2009. Bed bugs (*Cimex lectularius*) and clinical consequences of their bites. J. Am. Med. Assoc. 301: 1358–1366.

Goddard, J. and R. deShazo. 2012. Psychological effects of bed bug attacks (*Cimex lectularius* L.). Am. J. Med. 125: 101–103.

Gordon, J. R., M. H. Goodman, M. F. Potter, and K. Haynes. 2014. Population variation in and selection for resistance to pyrethroid-neonicotinoid insecticides in the bed bug. Sci. Rep. 4: 3836. Doi: 10.1038/srep03836.

Griffiths, D. 1980. The feeding biology of ant-lion larvae: prey capture, handling and utilization. J. Anim. Ecol. 49: 99–125.

Harlan, H. 2007. Bedbug control: challenging and still evolving. Outlooks on Pest Manag. 18: 57–61.

Harlan, H., M. Faulde, and G. Baumann. 2008. Bed bugs. pp. 131–154 In: Bonnefoy, X., H. Kampen, and K. Sweeney (eds.), Public Health Significance of Urban Pests. Copenhagen, Denmark, World Health Organisation Europe.

Harraca, V., C. Ryne, and R. Ignell. 2010. Nymphs of the common bed bug (*Cimex lectularius*) produce anti-aphrodisiac defense against conspecific males. BMC Biology 8: 121.

Haynes, K. F. 2008. The secret life of bed bugs: sublethal effects of insecticides on bed bug behavior. PestWorld (Mar/Apr): 5–8.

Haynes K. F., and M. F. Potter. 2013. Recent progress in bed bug management. pp. 269–278. In: Ishaaya, I., S. R. Palli, and A.R. Horowit (eds.), Advanced Technologies for Managing Insect Pests. Springer Science & Business Media Netherlands.

Henry, T. J. 2009. Biodiversity of the Heteroptera. In: R. G. Foottit and P. H. Adler (eds) Insect biodiversity: Science and society. Wiley-Blackwell, Oxford.

Hosokawa, T., R. Koga, Y. Kikuchi, X. Meng, and T. Fukatsu. 2010. Wolbachia as a bacteriocyte-associated nutritional mutualist. Proc. Natl. Acad. Sci. U.S.A. 107: 769–774.

How, Y. F., and C. Y. Lee. 2010. Effects of life stages and feeding regimes on active movement behavior of the tropical bed bug, *Cimex hemipterus* (Hemiptera: Cimicidae). J. Med. Entomol. 47: 305–12.

Hwang S. W., T. J. Svoboda, I. J. De Jong, K. J. Kabasele, and E. Gogosis. 2005. Bed bug infestations in an urban environment. Emerg. Infect. Dis. 11: 533–538.

Johnson, C. G. 1937. The relative values of man, mouse and domestic fowl as experimental hosts for the bed-bug, *Cimex lectularius* L. Proc. Zool. Soc. London A 107: 107–126.

Johnson C. G. 1941. The ecology of the bed-bug, *Cimex lectularius* L., in Britain. J. Hyg. 41: 345–461.

Johnston, J. M. 1999. Canine detection capabilities: operational implications of recent R&D findings. Institute for Biological Detection Systems. Auburn University, Auburn, Alabama.

Jones S. C., and J. L. Bryant. 2012. Ineffectiveness of over-the-counter total-release foggers against the bed bug (Heteroptera: Cimicidae). J. Econ. Entomol. 105: 957–963.

Kells, S. A. 2006. Nonchemical control of bed bugs. Am. Entomol. 52: 109–110.

Kells, S., and M. Goblirsch. 2011. Temperature and time requirements for controlling bed bugs (*Cimex lectularius*) under commercial heat treatment conditions. Insects 2: 412–422.

Kemper, H. 1930. Contribution to the biology of *Cimex lectularius*. I. The effect of food deficiency. Z. Morphol. Oekol. Tiere. 19: 160–183.

Kemper, H. 1936. Die Bettwanze und ihre Bek¨ampfung. Hyg. Zool. 4: 1–107.

Kilpinen, O., M. Kristensen, and K. M. V. Jensen. 2011. Resistance differences between chlorpyrifos and synthetic pyrethroids in *Cimex lectularius* population from Denmark. Parasitol. Res. 109: 1461–1464.

Koganemaru, R., and D. M. Miller. 2013a. The bed bug problem: Past, present, and future control methods. Pesticide Biochem. Physiol. 106: 177–189.

Koganemaru, R., D. M. Miller, and Z. N. Adelman. 2013b. Robust cuticular penetration resistance in the common bed bug (*Cimex lectularius* L.) correlates with increased steady-state transcript levels of CPR-type cuticle protein genes. Pestic. Biochem. Physiol. 106: 190–197.

Krueger, L. 2000. Don't Get Bitten by the Resurgence of Bed Bugs. Pest Cont. 68: 58–64.

Lange, R., K. Reinhardt, N. Michiels, N. Anthes. 2013. Functions, mechanisms, and evolution of traumatic mating. Biol. Rev. 88: 585–601.

Lavoipierre, M. M. J. 1965. Feeding mechanisms of blood-sucking arthropods. Nature 208: 302–303.

Lehnert, M. 2013. Behavior and control of the bed bug *Cimex lectularius* L. (Hemiptera: Cimicidae). Doctoral dissertation, Clemson University. Available at: http://tigerprints.clemson.edu/cgi/viewcontent.cgi?article=2169&context=all_dissertations.

Leulmi, H., I. Bitam, J. M. Berenger, H. Lepidi, J. M. Rolain, L. Almeras, D. Raoult, and P. Parola. 2015. Competence of *Cimex lectularius* bed bugs for the transmission of *Bartonella qu*<u>**in**</u>*tana*, the agent of trench fever. PLoS Negl. Trop. Dis. 9: e0003789. Doi: 10.1371/journal.pntd.0003789.

Levinson, H. Z. and A. R. Bar Ilan. 1971. Assembling and alerting scents produced by the bedbug *Cimex lectularius*. Experientia. 27: 102–103.

Levinson, H. Z., A. R. Levinson, and U. Maschwitz. 1974. Action and composition of alarm pheromone of bedbug *Cimex lectularius* L. Naturwissenschaften 61: 684–685.

Lewis, V. R., C. F. Fouche, and R. L. Lemaster. 1997. Evaluation of dog-assisted searches and electronic odor devices for detecting the western subterranean termite. Forest Prod. J. 47: 79–84.

Lewis, V. R., S. E. Moore, R. L. Tabuchi, A. M. Sutherland, D. H. Choe, and N. D. Tsutsui. 2013. Researchers combat resurgence of bed bug in behavioral studies and monitor trials. Calif. Agric. 67: 172–178.

Lilly, D.G., S. L. Doggett, M. P. Zalucki, C. Orton, and R. C. Russell. 2009. Bed bugs that bite back, confirmation of insecticide resistance in the common bed bug, *Cimex lectularius*. Prof. Pest Manager 13(5): 22–24.

Lin, H. W. Chi, C. Lin., Y, Tseng., W. Chen., Y. Kung, and Y. Lien. 2011. Fire ant-detecting canines: a complimentary method in detecting red imported fire ants. J. Econ. Entomol. 104: 225–231.

Lit, L., and C. A. Crawford. 2006. Effects of training paradigms on search dog performance. Appl. Anim. Behav. Sci. 98: 277–292.

Lit, L., J. B. Schweitzer, and A. M. Oberbauer. 2011. Handler beliefs affect scent detection dog outcomes. Anim. Cogn. 14: 387–394.

Lowe, C. F., and M. G. Romney. 2011. Bedbugs as vectors for drug-resistant bacteria. Emerg. Infect. Dis. 17: 1132–1134.

Madge, O. 2011. European code of practice, bed bug management. Bed Bug Foundation, London, UK. Available at: www.bedbugfoundation.org/downloads/45632_BedBug_ECoP_v9_forWeb.pdf.

Mamidala, P., A. Wijeratne, S. Wijeratne, K. Kornacker, B. Sudhamalla, L. Rivera-Vega, A. Hoelmer, T. Meulia, S. Jones, O. Mittapalli. 2012. RNA-Seq and molecular docking reveal multi-level pesticide resistance in the bed bug. BMC Genomics 13: 6. Doi: 10.1186/1471-2164-13–6.

Marshal, A. G. 1981. The ecology of ectoparasitic insects. Ecol. Entomol. 6: 55–74.

Marx, R. 1955. Über die Wirtsfindung und die Bedeutung des artspezifischen Duftstoffes bei *Cimex lectularius* Linné. Zeitschrift für Parasitenkd. 17: 41–73.

Mellanby, K. 1932. Effects of temperature and humidity on the metabolism of the fasting (*Cimex lectularius* L.). Parasitol. 24: 419–428.

Mellanby, K. 1939. The physiology and activity of the bed-bug (*Cimex lectularius* L.) in a natural infestation. Parasitol. 31: 200–2011.

Millikan, L. E. 1993. Papular urticaria. Semin. Dermatol. 2: 53–56.

Moore, D. J., and D. M. Miller. 2009. Field evaluations of insecticide treatment regimens for control of the common bed bug *Cimex lectularius* (L.). Pest Manag. Sci. 65: 332–338.

Morrow, E. H., and G. Arnqvist. 2003. Costly traumatic insemination and a female counter-adaptation in bed bugs. Proc. R. Soc. Lond. B. 267: 307–313.

National Pest Management Association 2011. Best management practices for bed bugs. Available at: http://npmapestworld.org/publicpolicy/documents/NPMABedBugBMP-10-22-11.pdf.

Naylor, R. A., and C. J. Boase. 2010. Practical solutions for treating laundry infested with *Cimex lectularius* (Hemiptera: Cimicidae). J. Econ. Entomol. 103:136 –139.

Naylor, R. 2012. Ecology and dispersal of the bedbug. Doctoral dissertation, University of Sheffield. Available at: http://etheses.whiterose.ac.uk/2244/1/Naylor%2C_Richard.pdf.

Newberry, K. and E. J. Jansen. 1986. The common bedbug *Cimex lectularius* in African huts. T. Roy. Soc. Trop. Med. Hyg. 80: 653–658.

Olson, J. F., R. D. Moon, and S. A. Kells. 2009. Off-host aggregation behavior and sensory basis of arrestment by *Cimex lectularius* (Heteroptera: Cimicidae). J. Insect Physiol. 55: 580–587.

Omori, N. 1941. Comparative studies on the ecology and physiology of common and tropical bed bugs, with special references to the reaction to temperature and moisture. J. Formos. Med. Assoc. 60: 555–729.

Oxley, J. C., and L. P. Waggoner. 2009. Detection of explosives by dogs. pp. 27–40. In: Marshall, M. and J. C. Oxley (eds.), Aspects of explosives detection. Elsevier, Amsterdam, The Netherlands.

Panagiotakopulu, E., and P. C. Buckland. 1999. *Cimex lectularius* L., the common bed bug from Pharaonic Egypt. Antiquity 73: 908–911.

Parsons, D. J. 1955. Bed bug bite anaphylaxis misinterpreted as coronary occlusion. Ohio Med. J. 51: 669.

Pereira, R. M., P. G. Koehler, M. Pfiester, W. Walker. 2009. Lethal effects of heat and use of localized heat treatment for control of bed bug infestations. J. Econ. Entomol. 102: 1182–1188.

Pereira, R. M., and P. G. Koehler. 2011. Use of heat, volatile insecticide, and monitoring tools to control bed bugs (Heteroptera: Cimicidae). pp. 325–329. In: Robinson WH, Bajomi D (ed.), Proceedings of the 7th International Conference on Urban Pests, Ouro Preto, Brazil, Instituto Biologico, Sao Paulo, Brazil.

Pereira, R. M., A. S. Taylor, M. P. Lehnert, and P. G. Koehler. 2013. Potential population growth and harmful effects on humans from bed bug populations exposed to different feeding regimes. J. Med. Vet. Entomol. 27: 148–155.

Pfiester, M., P. G. Koehler, and M. Pereira. 2008. Ability of bed bug-detecting canines to locate live bed bugs and viable bed bug eggs. J. Econ. Entomol. 101: 1389–1396.

Pfiester, M., P. G. Koehler, and R. M. Pereira. 2009a. Sexual conflict to the extreme: traumatic insemination in bed bugs. Am. Entomol. 55: 244–249.

Pfiester, M., P. G. Koehler, and R. M. Pereira. 2009b. Effect of population structure and size on aggregation behavior of *Cimex lectularius* (Hemiptera: Cimicidae). J. Med. Entomol. 46: 1015-1020.

Phillips, T. W., and J. E. Throne. 2010. Biorational approaches to managing stored-product pests. Annu. Rev. Entomol. 55: 375–397.

Pinto, L. J., R. Cooper, and S. K. Kraft. 2007. Bed bug handbook-the complete guide to bed bugs and their control. Pinto & Associates, Inc., Mechanicsville, MD.

Polanco, A. M., C. Brewster, and D. M. Miller. 2011a. Population growth potential of the bed bug, *Cimex lectularius* L.: a life table analysis. Insects 2: 173–185.

Polanco, A. M., D. M. Miller and C. Brewster. 2011b. Reproductive potential of field-collected populations of *Cimex lectularius* L and the costs of traumatic insemination. Insects 2: 326–335.

Polanco, A. M., D. M. Miller, and C. Brewster. 2011c. Survivorship during starvation for *Cimex lectularius* L. Insects 2: 232–242.

Potter, M. F. 2005. A bed bug state of mind: emerging issues in bed bug management. Pest Control Technol. 33(10): 82–85.

Potter, M. F. 2006. Perfect storm: an extension view on bed bugs. Am. Entomol. 52: 102–104.

Potter, M. F. 2011. The history of bed bug management-with lessons from the past. Am. Entomol. 57: 14–25.

Potter, M. F., A. Romero, K. F. Haynes, and W. Wickemeyer. 2006. Battling bed bugs in apartments. Pest Control Technol. 34(8): 44–52.

Potter, M. F., A. Romero, K. F. Haynes, E. Hardebeck. 2007. Killing them softly: battling bed bugs in sensitive places. Pest Control Technol. 35(1): 24–32.

Potter, M. F., A. Romero, and K. F. Haynes. 2008a. Battling bed bugs in the USA. pp. 400–406. In: W. H. Robinson and D. Bajomi (eds.), Proceedings of the 6th International Conference on Urban Pests, OOK-Press Kft., Hungary.

Potter, M. F., A. Romero, K. F. Haynes, E. Hardebeck, and W. Wickemeyer. 2008b. Is there a new bed bug answer? Pest Control Technol. 36(6): 116–124.

Potter M. F., K. F. Haynes, K, Connelly, K. M. Deutsch, E. Hardebeck, D. Partin, and R. Harrison. 2010. The sensitivity spectrum: human reactions to bed bug bites. Pest Control Technol. 38(2): 70–74, 100.

Potter, M. F., K. F. Haynes, B. Rosenberg, and M. Henrikson. 2011. The 2011 bed bugs without borders survey: defining the bed bug resurgence. PestWorld (Nov/Dec): 4–15.

Potter, M. F., K. F. Haynes, J. R. Gordon, E. Hardebeck, and W. Wickemeyer. 2012. Dual-action bed bug killers. Pest Control Technol. 40(3): 62, 63, 65–8, 75, 76.

Potter, M. F., K. F. Haynes, B. Rosenberg, and M. Henrikson. 2013a. Bed bug Nation: are we making any progress? Pestworld (Sept/Oct): 3–11.

Potter, M. F., J. R. Gordon, M. H. Goodman, and T. Hardin. 2013b. Mapping bed bug mobility. Pest Control Technol. 41(6): 72–74, 76, 78, 80.

Potter, M. F., K. F. Haynes, J. R. Gordon, L. Washburn, M. Washburn, and T. Hardin. 2014. Silica gel: A better bed bug desiccant? Pest Control Technol. 42(8): 76–81, 84.

Potter, M. F., K. F. Haynes, and J. Fredericks. 2015. Bed bugs across America: the 2015 bugs without borders survey. Pestworld (Nov/Dec): 4–14.

Pritchard, J. and S. W. Hwang. 2009. Severe anemia from bedbugs. Canad. Med. Assoc. J. 181: 287–288.

Puckett, R. T., D .L. McDonald, and R. E. Gold. 2012. Comparison of multiple steam treatment durations for control of bed bugs (*Cimex lectularius* L.). Pest Manag. Sci. 69: 1061–1065.

Pulliam, H. R., and T. Caraco. 1984. Living in groups: is there an optimal group size? pp. 122–147. In: J. R. Krebbs and N. B. Davies (eds.), Behavioural ecology: an evolutionary approach, 2nd ed. Sinauer, Sunderland, Mass.

Ralph N., H. E. Jones, and L. E. Thorpe. 2013. Self-reported bed bug infestation among New York City residents: prevalence and risk factors. J. Environ. Health 76:38–45.

Rao, H. 1972. Abnormal sexual behavior of isolated males of *Cimex lectularius* L. Ind. J. Exp. Biol. 10: 295–297.

Reinhardt K., R. Naylor, M. T. Siva-Jothy. 2005. Potential sexual transmission of environmental microbes in a traumatically inseminating insect. Ecol. Entomol. 30: 607–611.

Reinhardt, K., and M. T. Siva-Jothy. 2007. The biology of bedbugs (Cimicidae). Annu. Rev. Entomol. 52: 351–374.

Reinhardt, K., R. Naylor, M. T. Siva-Jothy. 2009a. Situation exploitation: higher male mating success when female resistance is reduced by feeding. Evol. 63:29–39.

Reinhardt, K., D. Kempke, R. A. Naylor, and M. T. Siva-Jothy. 2009b. Sensitivity to bites by the bedbug, *Cimex lectularius*. Med. Vet. Entomol. 23: 163–166.

Reinhardt, K., D. Isaac, and R. Naylor. 2010. Estimating the feeding rate of the bedbug *Cimex lectularius* in an infested room: an inexpensive method and a case study. Med. Vet. Entomol. 24: 46-54.

Reinhardt, K., N. Anthes, and R. Lange. 2014. Copulatory wounding and traumatic insemination. pp. 115–139. Cold Spring Harb. Perspect. Biol. Doi: 10.1101/cshperspect.a017582.

Reis, M. D., and D. M. Miller. 2011. Host searching and aggregation activity of recently fed and unfed bed bugs (*Cimex lectularius* L.). Insects 2: 185–194.

Rivnay, E. 1932. Studies in tropisms of the bed bug *Cimex lectularius* L. Parasitol. 24: 121–36.

Rivnay, E. 1933. Tropisms effecting copulation in the bed bug. Psyche 24: 121–36.

Robinson, W. H., C. J. Boase. 2011. Bed bug (Hemiptera: Cimicidae) resurgence: plotting the trajectory. pp. 315–318. In: Robinson WH, Bajomi D (ed.), Proceedings of the 7th International Conference on Urban Pests, Ouro Preto, Brazil. Instituto Biologico, Sao Paulo, Brazil.

Romero, A., M. F. Potter, and K. F. Haynes. 2007a. Insecticide resistant bed bugs: implications for the industry. Pest Control Technol, 35(7): 42–46.

Romero, A., M. F. Potter, D. Potter, and K. F. Haynes. 2007b. Insecticide resistance in the bed bug: a factor in the pest's sudden resurgence? J. Med. Entomol. 44: 175–178.

Romero, A., M. F. Potter, and K. F. Haynes. 2009a. Behavioral responses of the bed bug to insecticide residues. J. Med. Entomol. 46: 51–57.

Romero, A., M. F. Potter, and K. F. Haynes. 2009b. Are dusts the bed bug bullet? Pest Manag. Professional 77(5): 22–30.

Romero, A., M. F. Potter, and K. F. Haynes. 2010a. Circadian rhythm of spontaneous locomotor activity in the bed bug, *Cimex lectularius* L. J. Insect Physiol. 56: 1516–1522.

Romero, A., M. F Potter, and K. F. Haynes. 2010b. Evaluation of chlorfenapyr for control of the bed bug, *Cimex lectularius* L. Pest Manag. Sci. 66: 1243–1248.

Rossi L., and S. Jennings. 2010. Bed Bugs: A public health problem in need of a collaborative solution. Environ. Health 72: 34–35.

Ryckman, R. E., D. G. Bentley, and E. F. Archbold. 1981. The Cimicidae of the Americas and Oceanic Islands, a checklist and bibliography. Bull. Soc. Vector Ecol. 6: 93–142.

Ryne, C. 2009. Homosexual interactions in bed bugs: alarm pheromones as male recognition signals. Anim. Behav. 78: 1471–1475.

Saenz, V. L., W. Booth, C. Schal, and E. L. Vargo. 2013. Genetic analysis of bed bug populations reveals small propagule size within individual infestations but high genetic diversity across infestations from the eastern United States. J. Med. Entomol. 49: 865–875.

Salazar, R., R. Castillo-Neyra, A. W. Tustin, K. Borrini-Mayorí, C. Náquira, and M. Z. Levy. 2015. Bed bugs (*Cimex lectularius*) as vectors of *Trypanosoma cruzi*. Am. J. Trop. Med. Hyg. 92: 331–335.

SAS Institute. 2011. SAS/STAT user's guide, version 9.3. SAS Institute Inc., Cary, NC.

Savidge, J. A., J. W. Stanford, R. N. Reed, G. R. Haddock, and A. A. Yackel Adams. 2011. Canine detection of free-ranging brown tree snakes on Guam. N. Z. J. Ecol. 35: 174.

Schaafsma, E. J., S. D. Hapke, and M. G. Banfield. 2012. Bed bug (*Cimex lectularius* L.) population composition as determined by baited traps. Insects 3: 442–452.

Schal, C., and R. L. Hamilton. 1990. Integrated suppression of synanthropic cockroaches. Annu. Rev. Entomol. 35: 521–551.

Siljander, E., R. Gries, G. Khaskin, and G. Gries. 2008. Identification of the airborne aggregation pheromone of the common bed bug, *Cimex lectularius*. J. Chem. Ecol. 34: 708–718.

Singh, N., C. Wang, and R. Cooper. 2012. Interactions among carbon dioxide, heat, and chemical lures in attracting the bed bug, *Cimex lectularius* L. (Hemiptera: Cimicidae). Psyche. Doi: 10.1155/2012/273613.

Singh, N., C. Wang, and R. Cooper. 2013a. Effect of trap design, chemical lure, carbon dioxide release rate, and source of carbon dioxide on efficacy of bed bug monitors. J. Econ. Entomol. 106: 1802–1811.

Singh, N., C. Wang, and R. Cooper. 2013b. Effectiveness of a reduced-risk insecticide based bed bug management program in low-income housing. Insects 4: 731–742.

Singh, N., C. Wang, and R. Cooper. 2014. Potential of essential oil-based pesticides and detergents for bed bug control. J. Econ. Entomol. 107: 2163–2170.

Singh, N., C. Wang, and R. Cooper. 2015a. Effectiveness of a sugar-yeast monitor and a chemical lure for detecting bed bugs. J. Econ. Entomol. 108. Doi: 10.1093/jee/tov061.

Singh, N., C. Wang, and R. Cooper. 2015b. Role of vision and mechanoreception in bed bug, *Cimex lectularius* L. behavior. PLoS ONE 10: e0118855. Doi: 10.1371/journal.pone.0118855.

Siva-Jothy, M. T. 2006. Trauma, disease, and collateral damage: conflict in cimicids. Phil. Trans. R. Soc. B. 361: 269–275.

Smith, D. A., K. Ralls, A. Hurt, B. Adams, M. Parker, B. Davenport, M. C. Smith and J. E. Maldonado. 2003. Detection and accuracy rates of dogs trained to find scats of San Joaquin kit foxes (Vulpes macrotic mutica). Anim. Conserv. 6: 339–346.

Smith R., and H. T. Reynolds. 1965. Principles, definitions and scope of integrated pest control. Proceedings FAO Symposium Integrated Pest Control Rome: Food Agric. Organ. 1: 11–17.

Stedfast, M., and D. M. Miller. 2014. Development and evaluation of a proactive bed bug (Hemiptera: Cimicidae) suppression program for low-income multi-unit housing facilities. J. Integ. Pest Manag. 5. Doi: http://dx.doi.org/10.1603/IPM14003 E1-E7.

Stradling, D. J. 1970. The estimation of worker ant populations by the mark-release-recapture method: an improved marking technique. J. Anim. Ecol. 70: 575–591.

Stutt, A. D., and M. T. Siva-Jothy. 2001. Traumatic insemination and sexual conflict in the bed bug *Cimex lectularius*. Proc. Natl. Acad. Sci. USA 98: 5683–5687.

Suchy, J. T., and V. R. Lewis. 2011. Host-seeking behavior in the bed bug, *Cimex lectularius*. Insects 2: 22–35.

Susser, S. R., S. Perron, M. Fournier, L. Jacques, G. Denis, F. Tessier, and P. Roberge. 2012. Mental health effects from urban bed bug infestation (*Cimex lectularius* L.): a cross-sectional study. BMJ open 2(5): e000838. Doi: 10.1136/bmjopen-2012-000838.

Szalanski, A. L., J. W. Austin, J. A. McKern, C. D. Steelman, and R. E. Gold. 2008. Mitochondrial and ribosomal internal transcribed spacer 1 diversity of *Cimex lectularius* (Hemiptera: Cimicidae). J. Med. Entomol. 45: 229–236.

Tawatsin, A., U. Thavara, J. Chompoosri, Y. Phusup, N. Jonjang, C. Khumsawads, P. Bhakdeenuan, P. Sawanpanyalert, P. Asavadachanukorn, M. S. Mulla, P. Siriyasatien, and M. Debboun. 2011. Insecticide resistance in bedbugs in Thailand and laboratory evaluation of insecticides for the control of *Cimex hemipterus* and *Cimex lectularius* (Hemiptera: Cimicidae). J. Med. Entomol. 48: 1023–1030.

Ter Poorten, M. C., and N. S. Prose. 2005. The return of the common bedbug. Ped. Dermatol. 22: 183−187.

Titschack, E. 1930. Untersuchungen uber das Wachstum, den Nahrungsverbrauch und die Eierzeugung 3. *Cimex lectularius* L. Z. Morphol.Oekol.Tiere 17: 471–551.

Usinger, R. 1966. Monograph of Cimicidae. Thomas Say Foundation, Vol. 7. College Park, MD: Entomol. Soc. of Am.

Vaidyanathan, R., and M.F. Feldlaufer. 2013. Bed bug detection: current technologies and future directions. Am. J. Trop. Med. Hyg. 88: 619–625.

Venkatachalam, P. S. and B. Belavady. 1962. Loss of haemoglobin iron due to excessive biting by bed bugs. A possible aetiological factor in the iron deficiency anaemia of infants and children. Trans. R. Soc. Trop. Med. Hyg. 56: 218–221.

Waggoner, P. L., M. H. Jones, M. Williams, J. M. Johnston, C. C. Edge, and J. A. Petrousky. 1998. Effects of extraneous odors on canine detection. pp. 355–362. In: Proceedings SPIE vol. 3575, Enforcement and Security Technologies.

Wallner, W. E., and T. L. Elllis. 1976. Olfactory detection of gypsy moth pheromone and egg masses by domestic canines. Environ. Entomol. 5: 183–186.

Wang, C., and R. Cooper. 2011. Environmentally sound bed bug management solutions, pp. 44–63. In: P. Dhang (ed.), Urban Pest Management: An Environmental Perspective. CABI International.

Wang, C., and R. Cooper. 2012. The future of bed bug monitoring. PestWorld (Jan/Feb): 4–9.

Wang, C., M. M. Abou El-Nour, and G. W. Bennett. 2007. Controlling bed bugs in apartments – a case study. Pest Control Technol. 35(11): 64, 66, 68, 70.

Wang, C., T. Gibb, and G. W. Bennett. 2009. Evaluation of two least toxic integrated pest management programs for managing bed bugs (Heteroptera: Cimicidae) with discussion of a bed bug intercepting device. J. Med. Entomol. 46: 566–571.

Wang, C., K. Saltzmann, E. Chin, G. W. Bennett, and T. Gibb. 2010. Characteristics of *Cimex lectularius* (Hemiptera: Cimicidae) infestation and dispersal in a high-rise apartment building. J. Econ. Entomol. 103: 172–177.

Wang, C., W. Tsai, R. Cooper, and J. White. 2011. Effectiveness of bed bug monitors for detecting and trapping bed bugs in apartments. J. Econ. Entomol. 104: 274–278.

Wang C., K. Saltzmann, T. Gibb, and G. Bennett. 2012. Comparison of three bed bug management strategies in a low-income apartment building. Insects 3: 402–409.

Wang, C., N. Singh, R. Cooper, C. Liu, and G. Buczkowski. 2013. Evaluation of an insecticide dust band treatment method for controlling bed bugs. J. Econ. Entomol. 106: 347–352.

Wang, C., N. Singh, R. Cooper. 2014. What causes bed bug control failure - the resident factor. Pest Control Technol. 42(8): 86–95.

Weeks, E. N. I., M. A. Birkett, M. M. Cameron, J. A. Pickett, and J. G. Logan. 2010. Semiochemicals of the common bed bug, *Cimex lectularius* L. (Hemiptera Cimicidae), and their potential for use in monitoring and control. Pest Manag. Sci. 67: 10–20.

Weeks, E. N. I., J. G. Logan, S. A. Gezan, C. M. Woodcock, M. A. Birkett, J. A. Pickett, and M. M. Cameron. 2011. A bioassay for studying behavioural responses of the common bed bug, *Cimex lectularius* (Hemiptera: Cimicidae) to bed bug-derived volatiles. Bull. Entomol. Res. 101: 1–8.

Weidner H. 1958. Die Entstehung der Hausinsekten. Z. Angew. Entomol. 42: 429–47.

Welch, J. B. 1990. A detector dog for screwworms (Diptera: Calliphoridae). J. Econ. Entomol. 83: 1932–1934.

Wertheim, B., E-J. A. van Baalen, M. Dicke, and L. E. M. Vet. 2005. Pheromone-mediated aggregation in nonsocial arthropods: an evolutionary ecological perspective. Annu. Rev. Entomol. 50: 321–346.

Wong M., N. Vaidyanathan, and R. Vaidyanathan. 2013. Strategies for housing authorities and other lower-income housing providers to control bed bugs. J. Community Housing Dev. June: 20–28.

Yoon, K. S., D. H. Kwon, J. P. Strycharz, C. S. Hollingsworth, S. H. Lee, and J. M. Clark. 2008. Biochemical and molecular analysis of deltamethrin resistance in the common bed bug (Hemiptera: Cimicidae). J. Med. Entomol. 45: 1092–1101.

Zhu, F. J. Wigginton, A. Romero, A. Moore, K. Ferguson, R. Palli, M. F. Potter, K. F. Haynes, and S. R. Palli. 2010. Widespread distribution of knockdown resistance mutations in the bed bug, *Cimex lectularius* (Hemiptera: Cimicidae), populations in the United States. Arch. Ins. Biochem. Physiol. 73: 245–257.

Zhu, F., H. Gujar, J. R. Gordon, K. F. Haynes, M. F. Potter, and S. R. Palli. 2013. Bed bugs evolved unique adaptive strategy to resist pyrethroid insecticides. Sci. Rep. 3: 1456. Doi: 10.1038/srep01456.