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Chen Zha

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**DEVELOPING AND IMPLEMENTING NOVEL METHODS TO CONTROL
GERMAN COCKROACHES AND BED BUGS**

By

CHEN ZHA

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Changlu Wang

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ABSTRACT OF THE DISSERTATION

Developing and implementing novel methods to control

German cockroaches and bed bugs

by Chen Zha

Dissertation Director:

Changlu Wang

The German cockroach, *Blattella germanica* L. (Blattodea: Blattellidae), and the common bed bug, *Cimex lectularius* L. (Hemiptera: Cimicidae), are two common indoor pests of significant medical, economical, and social importance. In my studies, I evaluated several novel approaches for managing German cockroaches and bed bugs in apartment buildings. My researches involved both field and laboratory studies. In my first study, I determined the effect of a community-wide cockroach integrated pest management (IPM) on cockroach and pyrethroid residue reduction in a complex consisting of 258 units within 40 buildings in New Brunswick, New Jersey. At 7 mo after IPM implementation, 85% of the cockroach infestations found in the initial survey were eliminated. After 7 mo, the average number of detected pyrethroids decreased significantly from 6 ± 1 mean (\pm SEM) and 5 ± 1 to 2 ± 1 and 3 ± 1 in the kitchen and bedroom, respectively; the average concentrations of targeted pyrethroids residue decreased significantly from initial concentrations of 1.45 ± 0.39 and 3.31 ± 2.22 ng/cm² to 0.29 ± 0.17 and 0.80 ± 0.61 ng/cm² in the kitchen and bedroom, respectively. In my second study, I investigated the spatial distribution patterns of German cockroach

infestations in a 188-unit high-rise apartment building before and after building-wide IPM implementation, and determined the optimum cockroach monitoring method in apartments. I found that the cockroach infestations among the apartments within the building were not independently distributed at 0 mo; infestations facing each other across the hallway, sharing walls, and sharing ceiling/floor were correlated to each other. However, infestations at 12 mo after IPM implementation were independent from each other. New infestations at 6 and 12 mo after IPM implementation were independent from existing infestations. Compared to 2 d trap placement, 14 d trap placement not only had higher trap catch, but also detected more infestations; therefore a longer period of trap placement is recommended for cockroach monitoring. In my third study, I evaluated the toxicities of various essential oils, silicone oils, and paraffin oil against bed bugs. The LD₅₀ values of the most effective essential oil (blood orange), paraffin oil (C5-20 Paraffins), and the most effective silicone oil (Dodecamethylpentasiloxane) were 0.184 ± 0.018 , 0.069 ± 0.012 , and 0.036 ± 0.005 mg/bug, respectively. Direct spray of 1% water solution of 3-[Hydroxy (polyethyleneoxy) propyl] heptamethyltrisiloxane, the only silicone oil that mixes well with water, resulted in 92% bed bug mortality after 1 d. Results of this study indicated silicone oils and paraffin oil have the potential to be used as a safer alternative bed bug control materials. In my fourth study, I evaluated the effect of moxidectin, an anthelmintic drug, on bed bug feeding, development, fecundity, and mortality. High concentrations of moxidectin reduced the fecundity of bed bug females. One time feeding on rabbit blood containing 20 and 40 ng/ml moxidectin significantly reduced digestion rates and nymph molting rates. Therefore, moxidectin treatment is a

promising addition to the existing bed bug management tools if its use on human can be approved in the future.

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INTRODUCTION

Various arthropods thriving in urban environment negatively affect the inhabitants by damaging their belongings and property, causing health hazards, or affecting their quality of life. Among urban pests, the German cockroach, *Blattella germanica* L. (Blattodea: Blattellidae), and the common bed bug, *Cimex lectularius* L. (Hemiptera: Cimicidae), are two common indoor pests.

The German cockroach is a cosmopolitan cockroach species which has been fully domesticated and adapted to indoor life. Cockroaches inhabiting indoor environments such as apartments, schools, restaurants, and hospitals act as potential vectors of various pathogens including *Salmonella*, *Pseudomonas aeruginosa*, and *Klebsiella oxytoca* (Fotedar and Banerjee 1993, Kopanic Jr et al. 1994, Tachbele et al. 2006, Jalil et al. 2012). Cockroaches may also play an important role in facilitating the spread of antibiotic-resistant bacteria (Fathpour et al. 2003, Elgderi et al. 2006). Besides this, the major medical significance of cockroaches is triggering allergies and asthma. Cockroach-borne allergens are common in U.S. homes (Arruda et al. 2001, Huss et al. 2001, Cohn et al. 2006), and lower socioeconomic groups have higher risks of exposure and sensitization to cockroach allergens (Arruda et al. 2001).

The common bed bug is an obligate blood feeder, which delivers bites that cause various symptoms including itching, rashes, anxiety, sleeplessness, and anaphylaxis (Hwang et al. 2005, Delaunay et al. 2011, Goddard and de Shazo 2012). People's sensitivities to bed bug bites are different, varying from minor to severe symptoms; however, insensitive people can be sensitized after repeated exposure (Reinhardt et al. 2009, Minocha et al. 2016). Although bed bugs are not known to transmit diseases, there

are many candidate pathogens that might be potentially transmitted by bed bugs such as *Wolbachia*, Q fever, and Chagas disease (Delaunay et al. 2011). Bed bug infestations also cause economic loss to residents and property managers: professional bed bug treatments are expensive (Hwang et al. 2005, Rossi and Jennings 2010), and people often discard furniture and other personal belongs (Wang et al. 2010). Public tolerance to bed bugs is almost zero, and lawsuits associated with bed bugs are becoming common (Donaldson 2006). Individuals with bed bug infestations in homes often become victims of social injustice, and are denied access to health care and other social service because people afraid of getting bed bugs by serving them (Aultman 2013). In North America and Western Europe, bed bug infestations became rare during the second half of the 20th century (Ryan et al. 2002); however, a global resurgence of bed bugs have been occurring during recent years (Doggett et al. 2004, Potter 2006, Wang and Wen 2011, Davies et al. 2012).

Due to the significant medical, economic, and social impacts of German cockroaches and bed bugs, cockroach and bed bug management has been important components in both urban entomological research and the pest management industry. As a result of regulatory restrictions on many conventional insecticides such as DDT, carbamate, and organophosphate insecticide, the mainstay chemicals used for cockroach and bed bug control over the past decades has been pyrethroid insecticides (Williams et al. 2008, Horton et al. 2011, Davies et al. 2012). Decades of conventional pesticide applications has resulted in high resistance levels to pyrethroids in cockroach and bed bug field populations (Valles 1998, Wei et al. 2001, Kristensen et al. 2005, Romero et al. 2007, Zhu et al. 2010, Davies et al. 2012). Besides the lack of effectiveness, conventional

sprays also pose risks in residents' health by exposing them to chemical residues (Whyatt et al. 2002, Quandt et al. 2004). Novel cockroach and bed bug control materials and methods are needed to improve the management of these pests.

Due to the need for effective and environmentally friendly methods to control urban pests, integrated pest management (IPM) has been emphasized in urban pest management during recent years. Instead of conventional pesticide sprays, various approaches are used in IPM implementation for cockroach and bed bug management such as monitoring, baiting, dusting, laundering, educating clients, as well as applying insecticides (Wang and Bennett 2006, Wang et al. 2009, Cooper et al. 2016). Compared to conventional pesticide treatments, IPM not only reduced the usage of pesticides, but also eliminated cockroach infestations more effectively (Miller and Meek 2004); IPM implementation in cockroach infested structures also significantly reduced cockroach allergen levels (Wang and Bennett 2006, Sever et al. 2007, Nalyanya et al. 2014). However, cockroach IPM is still not widely adopted by the public and the pest management industry. The documented benefit of building-wide IPM on pest and insecticide residue reduction is still very limited.

The **objectives** of my research projects are to: 1) evaluate the benefits of a building-wide cockroach IPM program, 2) investigate German cockroach infestation distribution patterns in apartment buildings, and 3) develop novel bed bug control methods.

In Chapter One, I designed and implemented a community-wide IPM program for cockroach management. The reduction of cockroach population and infestation rate was evaluated for 7 mo period after IPM implementation. Floor wipe samples were collected

before and after IPM to determine the number and concentration of target pyrethroid insecticides. The objectives of the study were to evaluate the efficacy of a community-wide cockroach IPM program, and to test the hypothesis that a significant reduction of indoor insecticide residues can be expected after IPM implementation.

In Chapter Two, I monitored the distribution of cockroach infestations in a high-rise apartment building over time. All cockroach infestations in the building were identified by sticky traps and treated by Rutgers researchers using IPM procedures. Building-wide inspections were repeated at 6 and 12 mo after initial inspection. The cockroach infestation distribution patterns before and after IPM implementation were compared, and the sensitivity of sticky traps placed for 2 d and 14 d for detecting cockroaches were compared. The objectives of the study were to determine the effect of building-wide IPM on the spatial distribution of cockroach infestations among apartments within a multi-unit building, and to discuss the efficacy of different monitoring methods.

In Chapter Three, I evaluated the toxicities of different essential oils, silicone oils, and paraffin oil against bed bugs. The toxicities were compared by topical assay, and then the LD₅₀ of selected oils were determined. The efficacy of a silicone oil-water mixture in killing bed bugs via direct spray was tested. The objectives of the study were to determine the toxicities of different oils against bed bugs, and to evaluate the feasibility of using silicone oil in bed bug control.

In Chapter Four, I determined the effect of moxidectin, an anthelmintic drug, on bed bug feeding, development, fecundity, and survivorship. Bed bugs were fed with blood containing different concentrations of moxidectin, and then observed for feeding rate, ingestion rate, digestion rate, female oviposition, egg hatching, nymph development,

and mortality. The objective of the study was to evaluate the direct and indirect effects of moxidectin on bed bugs.

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CHAPTER ONE

Evaluation of Community-wide Integrated Pest Management for Reducing Cockroach Infestations and Indoor Insecticide Residue

Abstract

Low-income apartments are often plagued with a high prevalence of pests. Conventional pest control service and residents' self-treatment for eliminating household pests, especially cockroaches, are not very effective. Frequent insecticide applications introduce indoor insecticide residues which pose potential health risks to residents. Integrated pest management (IPM) is recognized as more effective than chemical treatments. To reduce indoor environmental risks associated with pests and insecticides, I implemented a community-wide IPM program with the goal of reducing pest infestations and insecticide residues, and increasing resident satisfaction with pest control services. The study was carried out in a complex consisting of 258 units within 40 buildings in New Brunswick, New Jersey. Initial inspection found that the top three pests and their infestation rates to be: German cockroaches-28%, rodents-11%, and bed bugs- 8%. Immediately after the initial inspection, we implemented a cockroach IPM program and revisited treated apartments biweekly or monthly for 7 mo. Residents were interviewed before and after the program. Floor wipe samples were collected from selected apartments for pyrethroid residue analysis. The community-wide cockroach IPM program reduced cockroach counts per apartment by 96% 7 wk after initial treatment. At 7 mo after IPM implementation, 85% of the cockroach infestations found in the initial survey were eliminated. After 7 mo, the average number of detected pyrethroids decreased significantly from 6 ± 1 mean (\pm SEM) and 5 ± 1 to 2 ± 1 and 3 ± 1 in the kitchen and

bedroom, respectively; the average concentrations of targeted pyrethroids residue decreased significantly from initial concentrations of 1.45 ± 0.39 and 3.31 ± 2.22 ng/cm² to 0.29 ± 0.17 and 0.80 ± 0.61 ng/cm² in the kitchen and bedroom, respectively.

Introduction

The German cockroach (*Blattella germanica* L.) remains to be one of the most common indoor pests (Wang and Bennett 2006). Cockroaches are carriers and potential vectors for various pathogens such as *Salmonella*, *Pseudomonas aeruginosa*, *Klebsiella oxytoca*, etc. (Fotedar and Banerjee 1993, Kopanic Jr et al. 1994, Jalil et al. 2012). They also produce allergens. Huss et al. (2001) found that 21.5% of the homes sampled in Boston had detectable levels of cockroach allergens. Cohn et al. (2006) concluded that cockroach allergen concentrations from 11% of U.S. living room floors and 13% of kitchen floors exceeded the level of allergic sensitization. Among all the residents, lower socioeconomic groups had higher risks of exposure and sensitization to cockroach allergens (Arruda et al. 2001). While intense house cleaning will reduce cockroach allergens (Sarpong et al. 1996, Eggleston et al. 1999), it is also necessary to reduce cockroach infestations, the source of cockroach allergens. Sever et al. (2007) successfully reduced indoor cockroach allergen levels after 12 mo by applying cockroach gel baits alone. Therefore, effective cockroach management is very important for protecting residents' health and improving their quality of life, especially in low-income communities.

Besides cockroaches, bed bugs and rodents are also commonly found in multi-unit dwellings, especially those occupied by low-income residents (Bradman et al. 2005, Cooper et al. 2016, Wang et al. 2016). Bed bugs are nuisance blood feeders which have

had a global reemergence during the last decade (Davies et al. 2012). Rodents and cockroaches are important public health pests; in addition to mechanical transmission of diseases, rodents also cause damage to property, food loss, and like cockroaches, produce allergens (Phipatanakul 2002). Community-wide pest surveys are important ways to identify pest infestations; although some data from previous studies are available, there is a need for community-wide surveys of pest infestations to provide scientific data for more effective pest management in multifamily housing communities. Surveys for bed bugs and rodents were also included in our study, to investigate the prevalence of different indoor pest infestations in low-income communities.

Historically, conventional insecticide spray treatments caused development of insecticide resistance among cockroach populations. Resistance to various insecticides, such as organochlorine, organophosphate, and pyrethroid insecticides (Scott et al. 1990), has been widely spread in German cockroach field populations after being treated by conventional insecticide sprays for decades. Resistance to pyrethroids is also common in wild German cockroach population. Umeda et al. (1988) determined the susceptibility of a field-collected cockroach population to 12 insecticides, and found that they exhibited resistance to all pyrethroids tested and DDT. Atkinson et al. (1991) found a field-collected German cockroach strain to be highly resistant to 10 pyrethroid insecticides. More examples of resistance to pyrethroids in German cockroaches have been reported by other researchers (Wei et al. 2001, Fardisi et al. 2017, Wu and Appel 2017). Despite the prevalence of resistance among cockroach populations, pyrethroid sprays are still the most widely available class of pesticides to consumers and are commonly used by residents in eliminating cockroach and other indoor pest infestations due to their low cost

and convenience. A population-based survey found that a total of 254 (55%) surveyed households used insecticides (sprays, foggers, and other products) indoors for self-application (Bennett et al. 2011). Horton et al. (2011) found that spray cans were the most common residential insecticide products in stores in New York City, and pyrethroids were the most common insecticide class in spray formulations. The use of pyrethroids appeared to increase in consumer products after the residential restriction of organophosphorus insecticides in 2000-2001 (Williams et al. 2008, Horton et al. 2011).

Besides lacking effectiveness due to resistance, indoor application of insecticide sprays and foggers also poses risks of human exposure to insecticide residues. Indoor insecticide residues and their potential risks to residents' health have drawn the attention of researchers (Nishioka et al. 2001, Quandt et al. 2004). The concentrations of pyrethrin and pyrethroids in air and their deposition on surfaces of floors and walls after an indoor application are high enough to trigger concerns of inhalation and skin resorption (Class and Kintrup 1991). Whyatt et al. (2002) surveyed the influence of residential insecticide use on women during pregnancy, and found that 100% of the monitored women had detectable levels of various insecticides including a pyrethroid, trans-permethrin; among all the insecticide applications in their homes, most ($\geq 90\%$) were for cockroaches.

Bait formulations (gel bait and bait stations) replaced pyrethroid sprays and became the dominant cockroach treatment materials after 1990's, which provided much higher efficacy compared to pyrethroid sprays (Appel 1990, 1992, Buczowski et al. 2001, Gondhalekar et al. 2011). Although behavioral and physiological resistance against gel bait have been reported in some cockroach populations (Silverman and Ross 1994, Wang et al. 2004), baiting is still an effective method to control cockroach infestations. In

a study by Sever et al. (2007), the median cockroach count from sticky traps in apartments treated by researchers using gel bait decreased from 426.5 to 0 by 6 mo. In contrast, the median cockroach count decreased from 308.5 to 56 in apartments treated by commercial contractors using hydramethylnon gel bait, insect growth regulators, and pyrethroids; and increased from 205.5 to 285 in the non-treated control group. Wang and Bennett (2006) found that baiting alone can reduce cockroach trap catch by $94.6 \pm 2.8\%$ at 16 wk after initial intervention, and that integrated pest management (IPM) implementation combining various methods including baiting, trapping, and education is most effective in eliminating cockroach infestations.

Besides higher levels of pest control, implementing IPM is expected to reduce insecticide use in the long run (Ehler 2006). Arora (2009) compared IPM and non-IPM pest management for major pests (leaf hopper, shoot and fruit borer, etc.) in okra and brinjal fields, and found less insecticide residues in IPM fields. An analysis of pesticide residue data show that insecticide residues on IPM-grown food are lower than conventional-grown ones (Baker et al. 2002). It is believed that IPM implementation in urban environment can also benefit the residents' health by reducing the use of insecticides (Brenner et al. 2003). Williams et al. (2005) found that the residue levels of organophosphate insecticides in public schools with an IPM cockroach management approach is lower than a conventional pesticide treated group. However, how IPM implementation affects indoor residue level of pyrethroids, the most common active ingredients in household insecticide products, is still unknown. In this study, we surveyed the pest infestation (cockroaches, bed bugs, and rodents) levels in a low-income community to determine the prevalence of not only cockroach but also other common

indoor pests in a low-income community, applied a cockroach IPM program for 7 mo, and sampled for pyrethroids residues in 20 selected apartments before and after IPM implementation. We hypothesized that IPM implementation will not only eliminate most of the cockroach infestations within a 7 mo period, but also significantly reduce the presence and concentration of pyrethroid residues in apartments.

Materials and Methods

Study site and initial community-wide pest survey. An apartment complex managed by the New Brunswick Housing and Redevelopment Authority at New Brunswick, New Jersey was selected for this study. The building occupants were low-income families or seniors. The complex contained 258 units within 40 buildings, each building had one or two stories. As with many other low income communities in New Jersey, this community had disproportionally higher levels of pest infestations. The existing pest control provider hired by the housing authority came once a month to control pests based on a list of resident complaints provided by the management office. They would apply gel baits in kitchen cabinets for cockroach control and place glue boards for rodent control. If residents were not at home at the time of visit, the pest control technician would not enter the apartments. Based on our conversation, only about two tubes (60 g) of cockroach bait were used per month and about two technicians spent half to one day a month to service the entire complex.

We conducted community-wide household pest surveys in October 2015 and May 2016. A total number of 225 and 205 apartments were accessed during the two surveys, respectively. Other apartments were not accessed because they were vacant, had private locks installed by tenants, or we were refused access by the tenants. During the initial

survey, tenants who had ongoing indoor pest issues were asked the following questions: 1) Do you see any of the following pests in your apartment (rodents, cockroaches, or bed bugs)? 2) Have you used insecticides to control pests within the last 6 mo? 3) Are you satisfied with the pest control service provided by the pest control company? At 7 mo after IPM implementation, the same group of residents whose apartments were treated by the researchers were interviewed and asked the same questions. We also visually assessed the sanitation level (clean, normal, dirty) and clutter level (low, medium, high) in the kitchen each of these apartments in October 2015 and May 2016.

Each accessed apartment was inspected for signs of bed bug infestation in bedrooms and living rooms. If signs were present but no live bugs were found, ClimbUp[®] insect interceptors (Susan McKnight Inc., Memphis, TN) were installed under the bed and sofa legs, and inspected approximately two weeks later. Residents that were home during the inspections were asked if they sighted bed bugs.

Trapper[®] monitor & insect traps (1/3 of the 3 section trap) (Bell Laboratories Inc., Madison, WI) were placed in every apartment with confirmed or suspected cockroach infestation with signs such as cockroach feces, exuviae, or dead cockroaches. Six traps (labeled as 1~6) were placed in the kitchens and bathrooms and checked 1~4 days later, depending on our schedule and availability of housing staff for assistance in access to the apartments. The locations of the six monitoring traps were: 1) in kitchen cabinet under the sink, 2) in kitchen cabinet above the sink, 3) under the stove, 4) behind or beside the refrigerator, 5) on the kitchen floor next to the heater or trash can, and 6) behind toilet in the bathroom. All cockroach trap counts were adjusted to 1 day counts during analysis.

Insecticide residue floor wipes sampling. Twenty apartments with current pest infestations (18 with cockroaches and 2 with bed bugs) and insecticide use history within the previous 6 mo were chosen. The floor areas were wiped in accordance with the National Institute of Child Health and Human Development standard operating procedure for collection of pyrethroid residues (NICHD 2009). Two 0.3×0.3 m sample sites (929 cm² floor area) were selected in the kitchen and bedroom from each apartment. The locations were marked so that future samples could be taken from the exact same spots. Floor wipe samples were collected by MEDI-FIRST[®] 7.6 \times 7.6 cm gauze pads (Medique Products, Fort Myers, FL). Each gauze pad was added with 2 ml extraction solvent (70% ethanol) right before wiping. Using S-shaped, slightly overlapping motions, the wipe sample was collected first by wiping from left to right. The wipe was folded in half and used to wipe the same area in an up-and-down direction. The wipe was folded in half again and placed in a labeled straight-sided 20 ml amber glass jar sealed with a PTFE lined lid (Agilent Technologies, Santa Clara, CA). Samples were preserved in a cooler before arriving in the laboratory, and then stored under darkened conditions at -20°C until extraction (Deziel et al. 2011). Residents in all sampled apartments were provided with an educational handout on cockroach prevention and control by Rutgers researchers, and were encouraged to stop using pesticides on their own. At the same time, the apartments were treated for cockroaches by Rutgers researchers using IPM methods. Floor wipe samples were collected again in the same manner and from the exact same locations at 7 mo. The sampling areas were chosen in spots that were regularly disturbed by the residents' activities such as walking and cleaning, so the influence of 1st floor

wiping to 2nd sampling was neutralized. Dirty and dusty spots were avoided and not chosen as sampling areas.

The 1st sampling was taken in October 2015 (monthly mean temperature = 12.7°C), and the 2nd sampling was taken from the exact location as in the 1st sample in May 2016 (monthly mean temperature = 16.1 °C) (<http://weathersource.com/>). There were no controls in this study since all cockroach infestations had to be treated following the same protocol, and all bed bug infested apartments were scheduled to be treated by the contractor except the two included in residue study, which were treated by Rutgers researchers.

Ten target pyrethrins and pyrethroids commonly used in insecticide formulations were included in the spike samples using 5 commercial standard solutions or mixtures: pyrethrins & pyrethroids mixture #1 (cyfluthrin, d-(cis-trans)-phenothrin, fenvalerate, and tetramethrin), pyrethrins & pyrethroids mixture #2 (allethrin, permethrin, and resmethrin), λ -cyhalothrin, deltamethrin, and cypermethrin (Chem Service Inc., West Chester, PA). Standard mixture solutions containing an amount of 10, 50, 200, 500, 1000, 3000, 6000, and 10000 ng spike pyrethroids were used as spiked samples. A mixture of acetone and hexane (13:3) was used as solvent for spiked samples.

Samples were analyzed by Dr. Brian Buckley and Dr. Ill Yang in their laboratory of the Environmental and Occupational Health Science Institute (EOHSI), Rutgers University. A Solid phase microextraction coupled gas chromatography/ion trap mass spectrometry (SPME-GC/ITMS) process was used in analyzing the samples: insecticides in wipes were analyzed by an automated SPME coupled GC/ITMS method after a slight modification from a previously published method (Menezes et al. 2010). A 60 μ m

Polydimethylsiloxane/Divinylbenzene StableFlex fiber (Supelco, Bellefonte, PA) installed in a CTC Analytics Combi PAL system with SPME agitator attachment (Zwingen, Switzerland) was used for head space extraction (at 75 °C for 1 hour, 350 rpm) of insecticides as well as injection into a septum programmable injector (Varian 1079) operated in the splitless mode at 290°C. A microbore capillary GC column (DB × LB 40 m length, 0.18mm ID × 0.18µm film, Agilent Technologies, Santa Clara, CA) was used for all insecticide separations with a Varian CP-3800 gas chromatography system (Walnut Creek, CA). Elution of the analytes from the GC column occurred through a temperature program that ranged from 90°C to 320°C over a 40 min chromatographic run. Eluted insecticides were detected and analyzed by a Saturn 2200 ion trap mass spectrometer (Walnut Creek, CA), operated in EI positive mode after tuning with perfluorotributylamine (FC-43) according to the manufacture's manual. Data were collected using automatic gain control and optimized selected ion storage mode. The calibration standards were added to blank matrix extracts to correct for matrix background response enhancement.

IPM implementation. Immediately after the initial inspection of each apartment, we provided a one-page handout on cockroach prevention and control, insecticides and human health, and home pest control to each inspected apartment. For those infested with cockroaches, we orally instructed residents to clean the floors, reduce clutter, put away pet food during night, and stop using insecticides by themselves.

All homes with ≥ 10 cockroaches in monitoring traps during the first survey were treated with Advion[®] cockroach gel bait (Syngenta Crop Protection LLC., Greensboro, NC) and Borid[®] boric acid dust (Waterbury Companies Inc., Waterbury, CT). Cockroach

bait was applied mostly in kitchens and bathrooms where cockroaches were most prevalent. Boric acid dust was applied using a duster behind the refrigerator, stove, and toilet. We swept the area around the refrigerator using a broom before applying boric acid dust if it was dirty. The amount of insecticide used was based on the severity of the infestations.

For light infestations (<10 cockroaches in monitoring traps), we increased the number of traps instead of bait and insecticide dust. Six traps were placed in the same locations as the monitoring traps, and four more traps were added. They were placed on the kitchen counter, in the closet in the living room, and beside the refrigerator.

Each cockroach infested apartment was visited approximately every two weeks. During these visits, apartments were monitored by placing traps out using the same protocol as before. Traps were left in place for 1-4 d before being retrieved and examined. Additional treatment (baiting, dusting, placing new traps) was conducted depending on trap counts. If trap count was zero, the traps were left continuously in the apartments for detecting very low level infestations. Cockroach elimination was considered achieved when there were no cockroaches in traps over a 1 mo trapping period. The follow-up monitoring and retreatment lasted for 25 wk. Other non-target pests detected by the sticky traps were also recorded.

The two bed bug infested apartments included in insecticide residue sampling were treated by a combination of steaming, installing encasements on mattresses and box-springs, placing ClimbUp interceptors, and application of CimeXa insecticide dust (Rockwell Labs Ltd., North Kansas City, MO). All treatments were carried out by licensed Rutgers researchers. Residents were also educated by Rutgers researchers.

Residents were asked not to apply pesticides on their own and the pest control contractor was told not to apply pesticides in these two apartments. This was necessary to avoid any new insecticide applications by the pest control contractor.

For those apartments treated by Rutgers researchers, the existing pest control provider hired by the housing authority provided bed bug and rodent control in the cockroach infested units and rodent control only in the two bed bug infested apartments. The contractor visited the infested apartment on a monthly basis based on the list provided by the housing staff. They used Transport Mikron (active ingredient: acetamiprid, bifenthrin) (FMC Corporation, Philadelphia, PA) to treat bed bugs and rodent bait or glue boards for rodents. We advised the housing authority to request the contractor to adopt effective IPM strategies for managing bed bugs and rodents. A short list of cost-effective non-chemical and chemical bed bug control methods was provided to the contractor. However, based on our observations these recommendations were not adopted.

Statistical analysis. At 0 wk, all 6 traps were retrieved from 49 apartments, and the percentages of cockroaches caught in trap 1-6 were 4, 2, 36, 37, 17, and 4%, respectively. The ratio was used to adjust cockroach trap counts in apartments with missing traps. After that, the adjusted trap counts were divided by the number of days the traps were deployed and the number of infested apartments to calculate the average daily trap counts per infestation.

Insecticide samples between kitchen and bedroom at 0 mo and 7 mo were analyzed by all four possible comparisons (kitchen vs. bedroom, 0 mo vs. 7 mo) using paired t-test instead of two-way ANOVA, considering that kitchen and bedroom in the

same apartments were dependent to each other. The values of insecticide levels (ng/cm²) were log-transformed before analysis to meet the assumption of normality (Shapiro–Wilk test). All analyses were performed using SAS software version 9.3 (SAS Institute Inc. 2011). When comparing the residue levels of individual compound between kitchen and bedroom at 0 mo and 7 mo, the chemicals that were present in less than three apartments were not included.

Results

Pest infestation at 0 mo. Based on interviews and examination of monitoring traps at 0 mo, German cockroaches were the number one pest. A total of 62 apartments (28%) had German cockroaches. Rodents and bed bugs were the 2nd and 3rd most common household pests. Residents from 24 apartments (11%) reported rodent issues. A total number of 17 apartments (8%) were infested with bed bugs. Only seven residents (3%) mentioned having an ant issue in our survey. Only one resident complained about a termite issue in his apartment.

A total of 50 residents who thought they had ongoing pest infestations (cockroach, bed bug, and/or rodent) were interviewed. Among those residents, 44 (88%) used insecticides in the past 6 mo by themselves. Raid (0.06% imiprothrin + 0.10% cypermethrin, or other pyrethroids depending on product used) was the most frequently reported product (n = 24), followed by Hotshot (0.025% prallethrin + 0.100% cypermethrin, or other pyrethroids depending on product used) (n = 6), Combat bait (0.050% fipronil) (n = 3), boric acid (n = 2), Home Defense (0.0500% bifenthrin + 0.0125% zeta-cypermethrin) (n = 2), Bed Bug No More (1.0% clove oil + 0.6% cinnamon oil + 0.5% peppermint oil) (n = 1), Blackjack bed bug & flea killer (0.1%

pyrethrins) (n = 1), Harris Bed Bug Kit (0.03% deltamethrin) (n = 1), and Safer Ant & Crawling Insect Killer (diatomaceous earth) (n = 1). Nine of the interviewed residents did not remember the name of the products used.

Effect of IPM on cockroach infestation reduction. A total of 64 apartments were treated for cockroaches. This includes the initial 62 identified apartments and two more apartments subsequently added during the treatment process upon request by tenants at wk 9 and 19. After our treatment, the average cockroach count (based on a 1 day trapping period) per infested apartment (cockroach trap count > 0) decreased rapidly (Fig. 1). The mean count was reduced by 61, 77, 88, 87, and 96% at wk 2, 4, 7, 9, and 29, respectively. The slight increase in mean trap count at 9 wk was due to a new infested apartment being added to the list. During the 7-mo period, an average amount of 37 ± 5 g bait (1.2 tubes) and 36 ± 3 g dust were used in each treated apartment. The average time spent per apartment per visit (including the time moving between apartments) ranged from 11-14 minutes (two researchers worked together most of the time) and was relatively constant throughout the study period.

Pest infestation at 7 mo. During our 7 mo survey, 16 apartments (8% infestation rate) were identified with cockroach infestations. Among them, 10 were newly found infestations and 6 were treated by us but were never eliminated. Out of the 10 new infestations found during the 7 mo survey, 5 of them were not accessible during the 0 mo survey. Among the 64 units that were treated, cockroaches were eliminated in 46 apartments, 8 apartments still had cockroaches, and 12 apartments were not inspected during final survey due to lack of access. The confirmed elimination rate was 88% (46 out of 52). After 7 mo, 9 apartments had bed bug infestations (47% reduction), 20

apartments had rodent infestations (17% reduction). Among the 17 bed bug infestations found at 0 mo, 5 still had bed bugs, 8 were eliminated, and 4 were either refused access or vacant; therefore the confirmed elimination rate of bed bug infestations was 62% (8 out of 13).

A total of 21 residents whose apartments had been treated by Rutgers researchers were available for interview at both 0 and 7 mo. At 0 mo, 91% of the 21 residents said they used over-the-counter insecticides including sprays, insect bombs, baits, and dusts; and only 29% of the residents were satisfied with the existing pest control service provided by the management office. At 7 mo, 14% of the residents still saw traces of cockroach activity, and 91% of the residents were satisfied with our cockroach control service. Four residents used pesticides after we initiated the IPM program. They used Raid, boric acid, rodent bait, or “bed bug spray”.

We compared the sanitation level (clean, normal, dirty) and clutter level (low, medium, high) at 0 and 7 mo in the 21 apartments. At 7 mo, 5 apartments got better ratings in sanitation or clutter levels, 5 apartments got worse ratings, 9 apartments were the same as before, and 2 apartments were not evaluated. Besides the ratings, we did not observe an obvious overall improvement of the sanitation and clutter in the treated apartments.

Non-target pests detected by sticky traps. Non-target pests were recorded during 7 mo period cockroach IPM implementation from the 64 infested apartments. The most frequently recorded non-target indoor pest in cockroach monitoring traps was spiders (25.5%), followed by ants (19.7%), fungus gnats (10.6%), rodents (4.7%), sow bugs and pill bugs (4.4%), drain flies (4.0%), grain beetles (4.0%), bed bugs (2.9%),

spider beetles (2.9%), fruit flies (2.9%). Other miscellaneous non-target creatures found in traps included a silverfish, a parasitoid wasp, a demestid beetle, an aphid, fly, a spring tail, a weevil, a phorid fly, a carabid beetle, a house centipede, a crane fly, a millipede, a mite, a midge, an earwig, a cigarette beetle, a lady bug, a termite, a mealworm, and a cloth moth.

Insecticide residue in floor wipes. All of the residents in the 20 sampled homes used pesticides within the past 6 mo, 13 residents provided the names of the insecticides used. Among these residents, 10 residents used Raid spray. Other pyrethroid sprays used included Home Defense and Blackjack, each was used by one resident.

Floor wipe samples were collected again at 7 mo from 17 out of the 20 apartments sampled at 0 mo; the other three apartments were not accessible during the 2nd visit. At each sampling period, 10 pyrethroids were detected in floor wipe samples including isomers (Fig. 2). Among them, cyfluthrin was only present at 0 mo, and fenvalerate was only present at 7 mo. Resmethrin (isomer 1) was the most frequently found chemical at 0 mo which was present in 100% of the sampled apartments, followed by resmethrin (isomer 2) (94.1%) and allenthin (82.4%); those were still the three most commonly found chemicals at 7 mo, with a frequency of 82.4, 47.1, and 47.1%, respectively (Fig. 2).

At 0 mo, an average number of 6 ± 1 (mean \pm SE) insecticides were detected from the kitchen of each apartment, and 5 ± 1 chemicals were found in bedroom. There was no significant difference of number of chemicals detected in kitchen and bedroom at 0 mo ($t = 1.31$; $df = 16$; $P = 0.207$). At 7 mo, an average number of 2 ± 1 insecticides were detected from the kitchen of each apartment, and 3 ± 1 chemicals were found in bedroom. There was no significant difference of number of chemicals detected in kitchen and

bedroom at 7 mo ($t = -1.69$; $df = 16$; $P = 0.111$). There was significantly less number of chemicals detected in the 2nd sampling, both in kitchen and bedroom (Kitchen: $t = 4.9$; $df = 16$; $P = 0.002$. Bedroom: $t = 3.57$; $df = 16$; $P = 0.003$) (Fig. 3).

At 0 mo, mean concentration of pyrethroid residue on kitchen and bedroom floors was 1.45 ± 0.39 and 3.31 ± 2.22 ng/cm², respectively (Fig. 4). They were not significantly different ($t = -0.49$; $df = 16$; $P = 0.631$). At 7 mo, mean concentration of pyrethroid residue on kitchen and bedroom floors was of 0.29 ± 0.17 and 0.80 ± 0.61 ng/cm², respectively (Fig. 4). There was no significant difference in residue concentration between kitchen and bedroom at 7 mo ($t = -0.92$; $df = 16$; $P = 0.371$). From 0 mo to 7 mo, pyrethroid residues in kitchen decreased significantly by an average of $90.0 \pm 3.7\%$ ($t = 6.84$; $df = 16$; $P < 0.001$); pyrethroid residues in bedroom also decreased significantly ($t = 3.78$; $df = 16$; $P = 0.002$). Two apartments had increased residues (increased by 729% and 1568%, respectively) in the bedrooms. Excluding these two sample, the rest of the bedroom samples all showed a decrease of residues overtime, with an average reduction rate of $88.3 \pm 3.9\%$.

There were no significant difference for each individual pyrethroid at 0 mo between kitchen and bedroom. At 7 mo, only resmethrin (isomer 1) had significantly higher level in the bedroom than in the kitchen ($t = -2.53$; $df = 16$; $P = 0.022$). The concentration of the following individual chemicals were reduced significantly in the kitchen from 0 mo to 7 mo: allethrin ($t = 3.01$; $df = 16$; $P = 0.008$), trans-permethrin ($t = 2.68$; $df = 16$; $P = 0.017$), phenothrin ($t = 2.34$; $df = 16$; $P = 0.033$), resmethrin (isomer 1) ($t = 5.69$; $df = 16$; $P < 0.001$), resmethrin (isomer 2) ($t = 3.56$; $df = 16$; $P = 0.003$), and tetramethrin ($t = 3.07$; $df = 16$; $P = 0.007$). The concentration of the following individual

chemicals were reduced significantly in the bedroom from 0 mo to 7 mo: allethrin ($t = 2.95$; $df = 16$; $P = 0.010$), resmethrin (isomer 1) ($t = 4.71$; $df = 16$; $P < 0.001$), and tetramethrin ($t = 3.07$; $df = 16$; $P = 0.016$).

Discussion

The community-wide cockroach IPM program reduced cockroach counts in infested apartments by 88% at the 4th visit (7 wk after initial treatment), which is similar to previous studies in low income communities (Wang and Bennett 2006, Wang et al. 2013). After 7 mo, 85% of the cockroach infestations found in the initial survey were eliminated, confirming that the program was highly effective in eliminating German cockroaches.

Follow-up monitoring is critical in confirming cockroach elimination. In this study, the average number of treatments before cockroach elimination was 7. The high elimination rate from this study was partially due to the follow-up service procedure. Period community-wide pest survey is valuable for identifying new and missed infestations from previous inspections. Annual community-wide inspection using monitoring traps would be important for exterminators and management offices. Although, the survey requires significant labor, it would benefit the community in the long-term through avoiding unnecessary treatment and identifying infestations early.

Sticky traps not only are reliable monitoring tools for German cockroach infestations, but also reveal the existence of a variety of other indoor pests. Among those non-target captures, spiders and ants were the most frequently encountered. Many rodents and bed bug infestations were also identified and reported by using sticky traps during cockroach monitoring, although more effective target-specific monitors are available for

rodents and bed bugs. Sticky traps were widely applied as standard monitors in previous studies on cockroach management (Miller and Meek 2004, Wang and Bennett 2006, Nalyanya et al. 2014). Our results support the use of sticky traps as a valuable IPM tool in indoor pest management. Pest management professionals should always use sticky traps during household pest control services instead of baiting or spraying only.

The IPM program implementation changed residents' behavior and attitude towards pest control. After 7 mo, the percentage of interviewed residents who used insecticides was reduced by 79% (from 91% to 19%), while resident satisfaction increased from 29% to 91%. On the other hand, education did not improve the overall sanitation and clutter level. Therefore, it is more difficult to change residents' housekeeping behavior than to asking them to stop using insecticides. One reason why residents stopped doing self-application was most likely due to the significant reduction in cockroach numbers. Similar findings were reported by Wang and Bennett (2009) in bait treatment group, but they observed improvement in sanitation in IPM group, which was likely due to different researchers and residents were involved in different studies.

Complete cockroach elimination from the whole community is difficult to achieve. During our 7 mo treatment period, cockroaches were never eliminated in 6 apartments, and 10 new infestations were found during the 2nd survey. Lack of cooperation was the major obstacle in eliminating cockroach infestations on a community-wide scale. During our treatment, 19% (12 out of 64) apartments were not accessible during at least one visit. Failing to access apartments caused delayed treatments. Despite our education on the importance of cleaning, some apartments were still dirty and cluttered at the end of the treatment. In a few apartments, structural damage such as broken cabinets or leaking

pipes provided ideal environment for cockroaches. They were sometimes not repaired in a timely manner. Disturbance or loss of monitoring traps was common during our treatment. Disturbed traps were usually found relocated, flipped, flattened, or wet. According to tenants' reports, some traps were thrown away mostly because children and pets played or chewed on them, or there were live cockroaches caught in the traps. When using sticky traps as monitoring tools in apartments, such factors should be considered when deciding where to place traps.

Before the insecticide residue sampling, most of the interviewed residents used one or two consumer products for pest control, which could not explain the diversity of insecticide residues in floor wipe samples. It was unlikely that those residues were all introduced by the contractors, since most of the sampled apartments did not have bed bug infestations, and the contractor only used bait for cockroach control. One possibility is that the residents used a variety of different consumer products, but had difficulties in recalling the names of all the products used. It was common that residents could not recall the product name precisely and use vague terms like "roach sprays" or "bomb" instead.

The reason for a sudden increase in insecticide residue levels in two of the bedroom sampling sites at 7 mo is not clear. Cockroach infestations were eliminated from those two apartments, and they were not known to have a bed bug infestation. Despite the two outliers, the diversity and levels of insecticides residues in the sampled apartments were reduced significantly in both the kitchen and bedroom after our treatment. The result suggested that a significant reduction in indoor insecticide residues could be expected after a half-a-year period of IPM implementation.

Based on the protocol of the study, there were no control apartments during residue sampling since we only sampled homes with ongoing cockroach infestations, and all cockroach infestations in the community had to be treated by researchers in the same way for the benefit of housing and residents. Therefore, future studies focusing on the dynamics of indoor insecticide residues with no treatment or with conventional pest control would help us further understand the role of IPM in reducing indoor insecticide residues.

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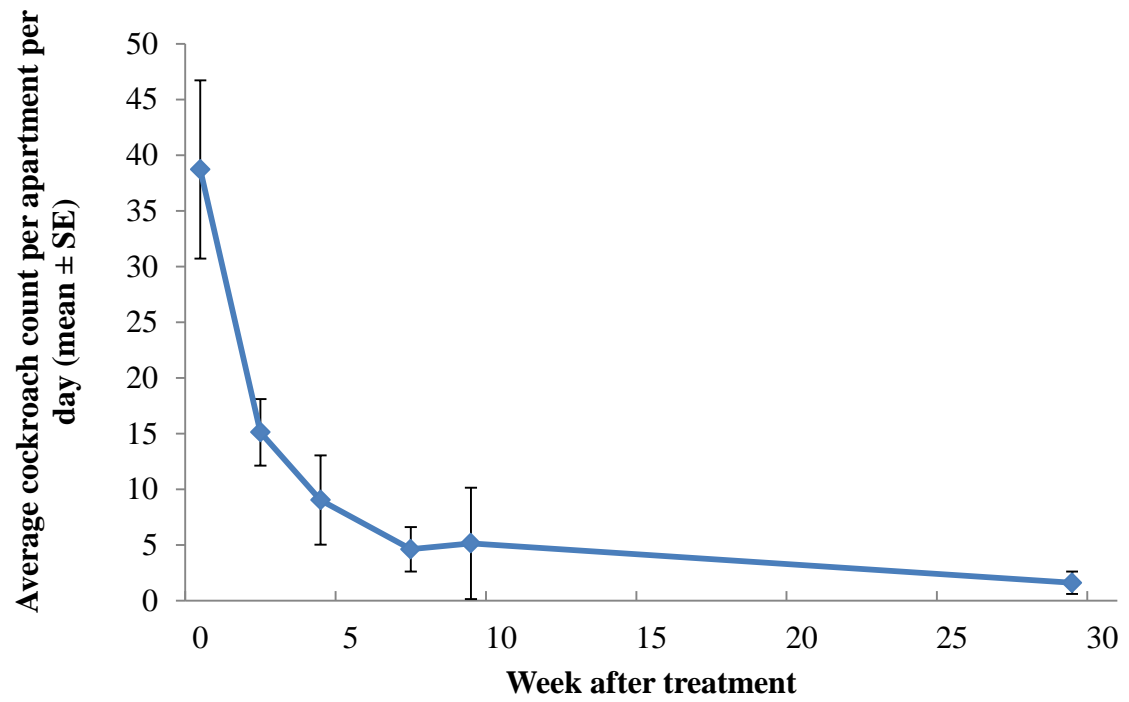


Fig. 1. Effect of IPM on cockroach count reduction in infested apartments. Only apartments with trap count > 0 were included in each monitoring period.

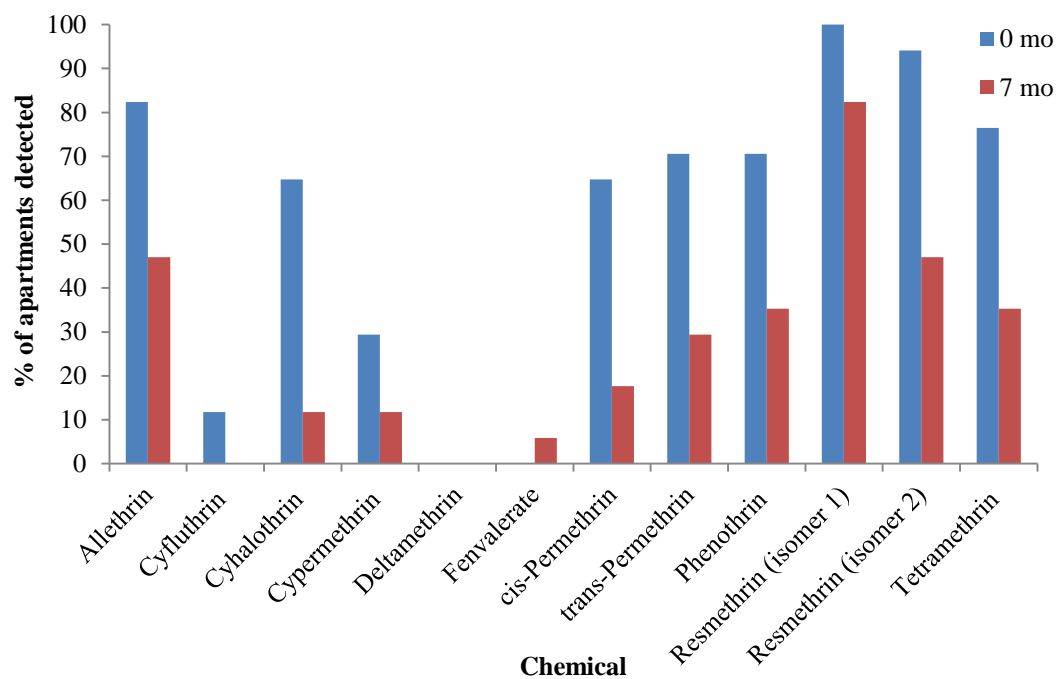


Fig. 2. Presence of insecticide residue in floor wipe samples before and after IPM implementation.

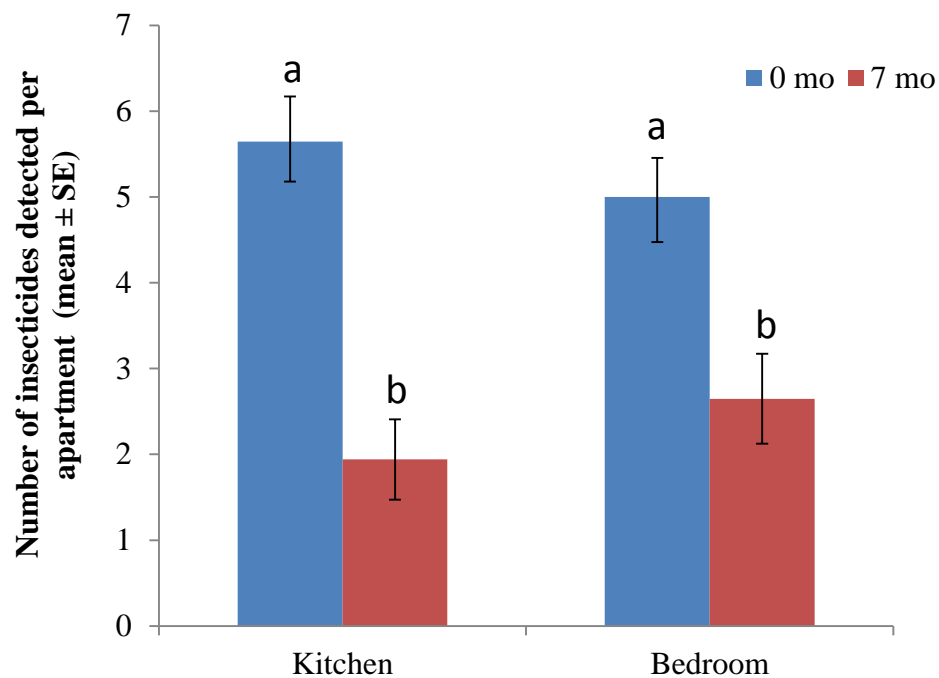


Fig. 3. Changes in the number of insecticides detected in kitchens and bedrooms before and after IPM intervention. Letters above each bar with different letters indicate significant difference (paired t-test, $P < 0.05$).

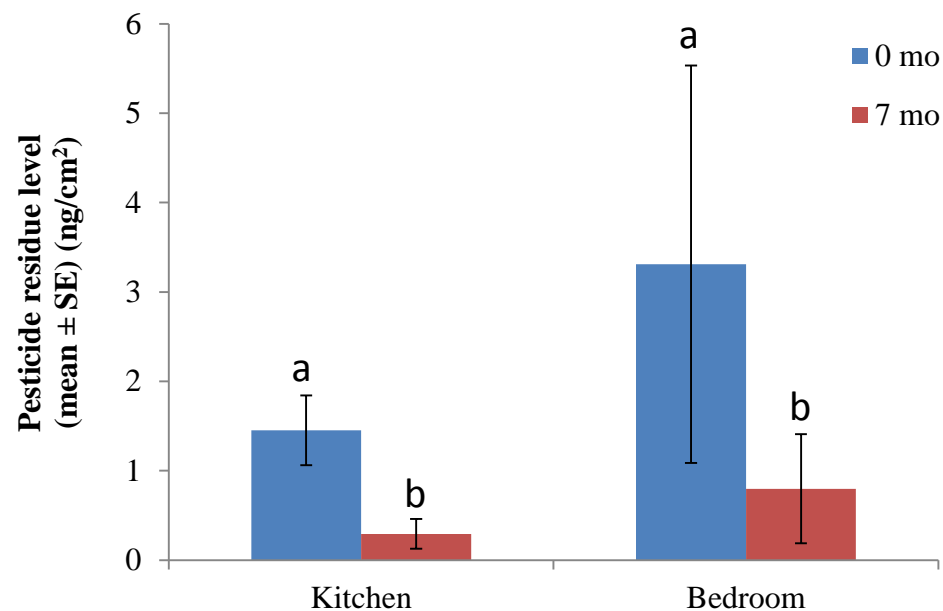


Fig. 4. Effect of IPM on insecticide residue concentrations in kitchens and bedrooms.

Letters above each bar with different letters indicate significant differences (paired t-test, $P < 0.05$). Statistical analysis was based on logarithmic transformed data.

CHAPTER TWO

Spatial Distribution and Monitoring of German Cockroaches in a High-rise Apartment Building

Abstract

The German cockroach (*Blattella germanica* L.) is one of the most common indoor pests. Our objectives are to investigate the spatial distribution patterns of German cockroach infestations in multi-unit dwellings before and after building-wide integrated pest management (IPM) implementation, and determine the optimum cockroach monitoring method. A high-rise apartment building with 188 residential units in Paterson, NJ was selected for building-wide cockroach inspection. All the identified infestations were treated by researchers using IPM techniques, and inspections were repeated at 6 and 12 mo after the initial survey. We examined the cockroach spatial distribution patterns within and between apartments, and compared the accuracy of two trap placement periods for detecting German cockroaches. Traps placed by the stove and refrigerator caught more cockroaches than traps placed in kitchen cabinets under the sink or in the bathroom. Cockroach infestations among the apartments within the building were not independently distributed at 0 mo; infestations facing each other across the hallway, sharing walls, and sharing ceiling/floor were correlated to each other. However, infestations at 12 mo after IPM implementation were independent from each other. Compared to 2 d trap placement, the 14 d trap placement not only had higher trap catch, but also detected more infestations. Building-wide cockroach IPM implementation not only reduced overall infestation rates, but also eliminated the correlation of infestations. Long-period (14 d) trap placement was more accurate in identifying low-level

infestations than a 2 d trapping period, and should be adopted in cockroach IPM instead of shorter periods.

Introduction

The German cockroach (*Blattella germanica* L.) is one of the most common indoor pests. German cockroaches in apartments, restaurants, hospitals, and other indoor environments spoil food, carry and distribute pathogens (Tachbele et al. 2006, Tilahun et al. 2012, Menasria et al. 2014), and play a significant role in triggering allergies and asthma by introducing cockroach-born allergens (Huss et al. 2001, Matsui et al. 2003, Cohn et al. 2006). Therefore, indoor cockroach management is important in improving the occupants' health and life quality.

German cockroaches are known for their aggregation and dispersal behavior within and among apartments. Rivault (1989) studied the spatial distribution of German cockroaches in a swimming-bath facility, and found that the distribution was contiguous: young nymphs explored as far as the border of the aggregation during development, while older nymphs and females remained in the shelter. Runstrom and Bennett (1990) found that German cockroaches dispersed among apartments, and 65% of the emigration came from adjoining kitchens. Crissman et al. (2010) analyzed the population genetic structure of German cockroaches in apartment buildings, and concluded that dispersal frequently occurred within the building but less common among building complexes. Within cockroach infested buildings, inter-apartment movement of up to 30% per week occurred where construction design permitted, and could be further promoted by pyrethrin insecticide applications (Owens and Bennett 1982). These findings strongly suggest that cockroach distributions between units within a building are not independent.

Considering the potential for dispersal of German cockroaches among neighboring units, building-wide integrated pest management (IPM) implementation is thought to be more effective in eliminating cockroach infestations in the whole structure (Wang and Bennett 2006). While IPM is very effective in reducing the infestations and cockroach numbers (Miller and Meek 2004, Wang and Bennett 2006), the effect of IPM on the spatial distribution of cockroach infestations is unclear. Studying the cockroach spatial distribution patterns before and after building-wide IPM implementation would help us understand how IPM affect cockroach infestation patterns and design appropriate strategies to eliminate remaining infestations.

Inspection and monitoring is a key element of an IPM program. The accuracy of the monitoring method adopted affects the success of IPM. Sensitivity of the monitoring method for detecting light infestations is especially important because failure to detect cockroaches when their numbers are low may result in delayed treatment or mistakenly elimination claims. A sticky trap is the most widely used monitoring tool in cockroach IPM implementation (Kakeh and Bennett 1997, Wang and Bennett 2006). In previous field studies, sticky traps were usually retrieved after 1-3 d (Miller and Meek 2004, Wang and Bennett 2006, Sever et al. 2007, Nalyanya et al. 2014). However, such a short period of time may not be sufficient to detect low infestations. Wang and Bennett (2006) found cockroaches in some apartments by visual inspection during follow-ups after IPM implementation, while no cockroaches were caught in traps after 1 d placement in the same apartments. During our previous cockroach IPM studies in low-income communities, we have observed similar situations that 1-2 d placement of traps failed to detect the existence of cockroaches while cockroaches were present in traps deployed for

1-2 wk. Although 1-3 d trap placement is widely accepted and used in previous cockroach IPM studies, it may not be the best monitoring method. Logically traps deployed for longer period will caught more cockroaches, but the possibility of failing to detected cockroach infestations by traps deployed for short period of time (1-3 d) is unknown. There is a need to determine the actual accuracy of short-period trap placement in detecting cockroach infestations compared to long-period trap placement.

The objectives of this study are to investigate the spatial distribution patterns of German cockroach infestations in multi-unit dwellings before and after building-wide IPM implementation, and compare the accuracy of two trap placement periods for detecting German cockroaches. We hypothesize that infestations in the same building were correlated to each other, but the correlation may be weakened after IPM implementation; and 14 d trap placement is more accurate and reliable in detecting cockroaches than 2 d trap placement.

Materials and Methods

Study site and initial building-wide cockroach monitoring. The study site was a high-rise apartment building managed by Joseph Masiello Homes (Paterson, New Jersey). The occupants were low-income seniors. The building contained 13 floors, 188 apartments. There were 15 apartments (14 one-bedroom and 1 two-bedroom apartments) on each floor except that only 8 one-bedroom apartments existed on the 1st floor. The other rooms on the 1st floor were used for offices and maintenance materials. Prior to our research project, the pest control in the building was done by a contracted pest management professional. The contractor stopped their monthly treatment service after our project started.

We conducted the initial building-wide cockroach monitoring in May 2016. Four Trapper[®] monitor & insect traps (1/3 of the whole piece) (Bell Laboratories Inc., Madison, WI) were placed in every apartment. The locations of the four traps were: 1) inside the kitchen cabinet under the sink, 2) beside the stove, 3) beside the fridge, and 4) beside the toilet in the bathroom. The traps were examined 14–17 d after installation. A total of 172 apartments were accessed and in which trap counts were recorded. The rest (16 apartments) were vacant, had private locks installed by the residents, declined receiving traps by residents, or all traps were thrown away by residents.

IPM implementation and cockroach monitoring between 0 and 6 mo. All German cockroach infested apartments received IPM treatment within one week after examining the traps. A one-page educational sheet on cockroach prevention and control was provided to residents with German cockroach infestations. If residents were at home during home visits, the researchers also orally instructed residents to clean the floors, reduce clutter, put away pet food during night, and stop using insecticides by themselves. All infestations were treated with a combination of Advion[®] Cockroach Gel Bait (0.6% indoxacarb, Syngenta Crop Protection LLC., Greensboro, NC) and Borid[®] dust (100% boric acid, Waterbury Companies Inc., Waterbury, CT). Cockroach bait was applied mostly in kitchens and bathrooms where most cockroach activity was detected. Boric acid dust was applied using a duster behind the refrigerator, stove, and toilet. Average amount of bait and boric acid dust applied per apartment during the initial treatment was 24 and 6 g, respectively.

The building was revisited 3 wk after the initial treatment. Four new traps were placed in the same locations. They were checked after 2 d and 12 d, respectively.

Apartments with > 5 cockroaches based on 2 d trap counts were re-treated with Advion bait. After the 3rd follow-up visits from the initial treatment, the 2 d trap catches were no longer recorded and all traps were examined biweekly only. Those traps that became dirty, missing, or having cockroaches were replaced with new traps during each visit. Additional bait was applied if the total trap count was ≥ 5 cockroaches during biweekly visits to infested apartments, and traps which became dirty, containing cockroaches, or missing were replaced with new traps during each visit. Alpine[®] Cockroach Gel Bait (0.5% dinotefuran, Whitmire Micro-Gen Research Laboratories, Inc., St. Louis, MO) was used at 17, 19, and 22 wk; and MaxForce[®] FC Select Roach Killer Bait Gel (0.01% fipronil, Bayer Environmental Science, Research Triangle Park, NC) was used at 24 and 26 wk. The rotation of different baits was to avoid resistance development in the cockroaches. Infestations were considered eliminated if no cockroach was found in traps during a 1-month period.

Second building-wide cockroach monitoring at 6 mo. The second building-wide cockroach monitoring was conducted in November 2016 (6 mo after the initial inspection). The apartments were monitored using the same method as the initial monitoring. Trap counts from 172 occupied apartments were obtained.

IPM implementation and cockroach monitoring between 6 and 12 mo.

Cockroach infestations identified during the 2nd building-wide inspection were monitored and treated on a monthly basis. Avert[®] DF Dry Flowable Cockroach Bait (0.05% abamectin, Whitmire Micro-Gen Research Laboratories, Inc., Saint Louis, MO) was used instead of gel baits to avoid development of resistance. Additional bait was applied if total trap count was ≥ 5 during monthly visits to infested apartments, and traps which

became dirty, contained cockroaches, or missing were replaced with new traps during each visit.

Final building-wide cockroach monitoring at 12 mo. The final building-wide cockroach monitoring was conducted in May 2017 (12 mo after the initial building-wide cockroach monitoring). All accessible apartments were monitored using the same method as the initial monitoring. Trap counts from 182 occupied apartments were obtained.

Data analysis. For traps placed for 14-17 days, the total cockroach trap catches within each apartment were adjusted to 14 d counts to standardize the measurement of cockroach population size. For traps placed for 2 d and 12 d consecutively during the first three follow-ups (5, 7, and 9 wk) after the initial treatment, 14 d trap catches were obtained by adding 2 d and 12 d trap catches. Percentage of cockroach counts in each trap location was calculated based on 67 apartments which had all four traps available during the initial survey. Then, the percentages were used for estimating trap catches in apartments with missing traps. Based on 14 d trap catch per apartment, infestations were categorized into three levels: light infestation (14 d trap catch 1-9), medium infestation (14 d trap catch 10-50), and heavy infestation (14 d trap catch > 50).

When analyzing the spatial pattern of infested units, infestation levels in apartments with no access at 0 mo were estimated based on 6 mo and 12 mo data. Three missed apartments at 0 mo were found to have ≥ 69 counts at 6 mo. Two of these apartments had cockroach activities at 0 mo but trap counts were not available. The infestation status of the other apartment was unknown. These three apartments were considered to have heavy infestations at 0 mo. All the other missed apartments at 0 mo

were considered as un-infested, since they had 0 or very few (≤ 8) cockroaches during 6 and/or 12 mo inspections.

All apartments without trap counts at 6 mo were considered as uninfested since they either had no cockroaches during 0 and 12 mo inspections, or had infestations at 0 mo but were treated until elimination. One apartment did not have cockroaches at 0 mo but was infested at 12 mo, we considered the apartment as uninfested at 6 mo because during 12 mo survey the resident said the infestation occurred recently. All apartments without trap count data at 12 mo were considered as uninfested since they were either treated until elimination or had no cockroaches during earlier inspections. Vacant units were considered as uninfested apartments. Non-residential units were excluded in the spatial analysis of the infestations. These units had no cockroach infestations based on monitoring traps placed in these units and staff observations.

The layout of the apartments is shown in Fig. 1. On each floor, every apartment has the entrance door facing to another across the hallway except one apartment in the middle part on each floor. For each floor except the 1st floor, there are three pairs of “across the hallway” neighbors in the left wing, one pair in the middle wing, and three pairs in the right wing. There are a total of 88 pairs of apartments neighboring to each other through “across the hallway”. For each pair of “across the hallway” neighbors, there were three possible infestation patterns (Fig. 2, top row of the diagram): both uninfested, one infested, and both infested.

On each floor of each wing, the three apartments on the same side of the hallway were adjoined to each other horizontally through shared walls. There are total of 5 sets of three apartments sharing walls on each floor (one in the left wing, one in the middle wing,

and one in the right wing). There are a total of 62 sets of three apartments sharing walls (except the 1st floor which only had two sets of three neighboring apartments). For each set of three apartments sharing walls, there are 6 possible infestation patterns (Fig. 2, 2nd and 3rd row). The 2nd row of Fig. 2 shows no infestation occurred in the middle apartment, its two neighbors were either both uninfested, one of the neighbors infested, or both neighbors infested. The 3rd row of Fig. 2 shows an infestation occurring in the middle apartment, its two neighbors were either both uninfested, one of the neighbors infested, or both neighbors infested.

Apartments up and downstairs adjoined to each other horizontally by shared ceilings/floors; in the case of no infestation occurring in the middle apartment, there are 3 possible infestation patterns: all uninfested, uninfested + one infested neighbor, and uninfested + two infested neighbors. In the case of infestation occurring in the middle apartment, there are 3 possible infestation patterns: infested + two uninfested neighbors, infested + one uninfested neighbor, and all infested. There are 158 groups of apartments sharing ceiling/floor with 2 neighbors in total, excluding the apartments on the 1st & 13th floor and 7 apartments on the 2nd floor which shared floor with non-residential units downstairs.

Based on infestation rate, we calculated the expected frequency of every single infestation pattern under the assumption that the infestations were independent. The difference between expected and observed frequency of distribution patterns was then compared by exact test of goodness-of-fit because some of the counts were small numbers (< 5). We also calculated the expected frequency of new infestations having at least one neighboring infestation 6 mo ago under the assumption that new infestations were

independent from existing ones, and compared with the observed frequency by exact test of goodness-of-fit.

Failure rate of 2 d traps compared to 14 d traps was calculated by dividing the number of infestations failed to be detected by 2 d traps by the number of infestations detected by 14 d traps.

The cockroach counts in each of the trap location 1-4 in heavily infested apartments (> 50 in total count per apartment) were compared by Analysis of Variance followed by Tukey's HSD test after passed Shapiro-Wilk test for normality test. The correlation between 2 d and 14 d trap catches were analyzed by linear regression. All statistical analyses were conducted using SAS software (version 9.3) (SAS Institute 2011).

Results

Spatial distribution of German cockroaches within apartments. Among all the apartments with cockroaches in the traps, 67 apartments had all four traps present during pick-up. A total number of 3,375 cockroaches were trapped, with 17, 35, 36, and 12%, in trap locations 1 to 4, respectively. These numbers were used for adjusting the total trap catches in apartments with missing traps.

Among those infested apartments, 18 had heavy infestations with median (min, max) trap counts of 52 (132, 514). The mean percentage of cockroaches found in trap 1 to 4 was 17 ± 4 , 34 ± 5 , 39 ± 5 , and $10 \pm 2\%$, respectively. The number of cockroaches caught in different locations was significantly different, with significantly more cockroaches caught in traps 2 and 3 than traps 1 and 4 ($F = 10.66$; $df = 3, 68$; $P < 0.001$. Tukey's HSD test, $P < 0.05$).

A total of 18 apartments had medium infestations with median (min, max) trap counts of 10 (22, 49). A total of 31 apartments had light infestations with median (min, max) trap counts of 1 (2, 7). Indoor distributions of cockroaches in medium and light infestations were not analyzed, since the data were not normally distributed even after transformation, and the distribution could be highly skewed when the trap catch is low.

Spatial distribution of German cockroach infestations in the building. During the initial survey at 0 mo, 14 d trap counts were obtained from 173 apartments. Cockroach infestations were detected in 89 apartments (51% infestation rate). Among those, 22 (25%) had heavy infestations, 25 (28%) medium infestations, and 42 (47%) light infestations. After adjustment of missed apartments, there were a total of 92 infestations out of 188 apartments (49% infestation rate) (Fig. 1.1).

Expected frequencies of different infestation patterns were calculated based on infestation rate. For example, the infestation rate at 0 mo was 49%; therefore, the possibility of an apartment to be uninfested is 51%. The expected frequency of two infestations being next to each other across the hall way is $0.49^2 \times 100\% = 24\%$, the expected frequency of two uninfested apartments being next to each other across the hall way is $0.51^2 \times 100\% = 26\%$, and the expected frequency of an uninfested apartments being next to an infestation across the hall way is $0.51 \times 0.49 \times 2 = 50\%$. The expected frequencies of other infestation patterns were calculated in the same way.

At 0 mo, the observed and expected infestation pattern frequency of apartments across the hallway was significantly different ($\chi^2 = 9.82$, $df = 2$, $P = 0.007$). The observed and expected infestation pattern frequency of apartments sharing walls was significantly different ($\chi^2 = 38.90$, $df = 5$, $P < 0.001$). The observed and expected infestation pattern

frequency of apartments sharing ceiling/floor was significantly different ($\chi^2 = 27.80$, $df = 5$, $P < 0.001$) (Table 1.1). These significant differences indicate that German cockroach distribution was not spatially independent. When one apartment was infested, its neighbors across the hallway, sharing the wall on the same floor, or sharing the ceilings were more likely to be infested than those without these spatial relationships.

During the second building-wide survey at 6 mo, 14 d trap counts were obtained from 172 apartments. German cockroaches were detected in 46 apartments (27% infestation rate). Among those, 5 (11%) had heavy infestations, 5 (11%) medium infestations, and 32 (78%) light infestations. After adjustment for missed apartments, there were a total of 46 infestations out of 188 apartments (24% infestation rate) (Fig. 1.2).

At 6 mo, the observed and expected infestation pattern frequency of apartments across the hallway was significantly different ($\chi^2 = 6.34$, $df = 2$, $P = 0.044$). The observed and expected infestation pattern frequency of apartments sharing walls was not significantly different ($\chi^2 = 6.09$, $df = 5$, $P = 0.302$). The observed and expected infestation pattern frequency of apartments sharing ceiling/floor was significantly different ($\chi^2 = 27.73$, $df = 5$, $P = 0.002$) (Table 1.2).

At 6 mo, 15 infestations were newly established, and 12 (80%) of them had at least one infested neighbor at 0 mo, which were not significantly different from expected frequency ($\chi^2 = 1.56$, $df = 1$, $P = 0.285$) (Table 2), indicating that the locations of new infestations were not associated with existing infestations.

During the final building-wide survey at 12 mo, 14 d trap counts were obtained from 182 apartments. German cockroaches were detected in 23 apartments (13%

infestation rate). Among those, 3 (13%) had heavy infestations, 5 (22%) medium infestations, and 15 (65%) light infestations. After adjustment for missed apartments, there were a total of 23 infestations out of 188 apartments (12% infestation rate) (Fig. 1.3).

At 12 mo, the observed and expected infestation pattern frequency of apartments across the hallway was not significantly different ($\chi^2 = 0.05$, $df = 2$, $P = 0.916$). The observed and expected infestation pattern frequency of apartments sharing walls was not significantly different ($\chi^2 = 3.79$, $df = 3$, $P = 0.267$); the number of uninfested and infested apartments with one or two infested neighbors were combined to avoid 0 counts in the statistical analysis. The observed and expected infestation pattern frequency of apartments sharing ceiling/floor was significantly different ($\chi^2 = 27.80$, $df = 5$, $P = 0.002$) (Table 1.3).

At 12 mo, 15 infestations were newly established, and 6 (40%) of them had at least one infested neighbor at 6 mo, which were not significantly different from expected frequency ($\chi^2 = 1.35$, $df = 1$, $P = 0.303$) (Table 2), indicating that the locations of new infestations were not significantly associated with existing infestations.

Correlation between 2 d and 14 d trap catch in German cockroach infested apartments. During 3-5 wk after initial treatment, the 2 d trap counts in cockroach infested apartments (with both 2 d and 14 d trap catch > 0) were positively correlated with 14 d trap catch ($F = 155.76$; $df = 1, 37$; $P < 0.001$. $R^2 = 0.81$) (Fig. 3.1). During 5-7 wk after initial treatment, the 2 d trap counts in cockroach infested apartments were positively but weakly correlated with 14 d trap counts ($F = 10.38$; $df = 1, 25$; $P = 0.004$. $R^2 = 0.30$) (Fig. 3.2). During 7-9 wk after initial treatment, the correlations between 2 d

and 14 d trap catch were not significant ($F = 2.17$; $df = 1, 14$; $P = 0.163$. $R^2 = 0.16$) (Fig. 3.3).

Comparison of 2 d placement and 14 d placement for detecting German cockroaches. During the 1st follow-up period (3-5 wk after initial treatment), 2 d and 14 d trap counts were both obtained from 83 apartments. The 14 d trap detected 56 infestations, while 2 d trap only detected 39 of those infestations. The 2 d placement failed to detect 30% of the infestations detected by 14 d placement.

During the 2nd follow-up period (5-7 wk after initial treatment), 2 d and 14 d trap counts were both obtained from 58 apartments. The 14 d trap detected 44 infestations, while 2 d trap only detected 27 of those infestations. The 2 d placement failed to detect 39% of the infestations detected by 14 d placement.

During the 3rd follow-up period (7-9 wk after initial treatment), 2 d and 14 d trap counts were both available from 47 apartments. The 14 d trap detected 31 infestations, while 2 d trap only detected 15 of those infestations. The 2 d trap failed to detect 48% of the infestations detected by 14 d placement (Table 3).

German cockroach detection from traps placed for extra-long period (6 mo). During the second building-wide inspection, traps deployed during the initial inspection were still present in 22 apartments with an average number of 2.1 ± 0.2 cockroach per trap. Among those apartments, 21 were confirmed as uninfested during both 0 and 6 mo inspection. However, cockroaches were present in traps deployed for 6 mo in 11 (52%) of those uninfested apartments, with an average number of 1.1 ± 0.3 cockroaches per trap.

Discussion

Using a building with large number of apartments and a long observation period, we found some interesting results on German cockroach spatial distribution patterns. Within each apartment, traps placed beside the stove and refrigerator caught a significantly higher proportion of cockroaches than traps placed under the sink and beside the toilet; traps at locations 2 and 3 caught 1.7 times more roaches than at locations 1&4. The result is similar to a previous study done by Nalyanya et al. (2014), with most cockroaches trapped in “food area” including areas under the kitchen sink and other kitchen counters, and in and around equipment such as refrigerators, freezers, and dishwashers; fewer cockroaches were caught under sink in our study, which may be caused by differences in house structures. The cockroach infestations within the building were spatially not independent: infestations among apartments across the hallway, sharing walls, and sharing ceiling/floor were correlated with each other. This conclusion is consistent with previous studies on the spatial distribution of cockroaches (Runstrom and Bennett 1990, Crissman et al. 2010).

At 0 mo, there was a trend that apartments across the hallway from each other were more likely to be both uninfested or both infested. The observed frequency of an infested apartment having an uninfested neighbor across the hallway was lower than expected. This suggests that cockroaches disperse to neighboring unit across the hallway. When there were three apartments adjoining to each other by shared walls, there was a trend that the observed frequencies for the three apartments being all uninfested or all infested were higher than expected, and the observed frequencies for an uninfested apartment having two infested neighbors or an infested apartment having two uninfested neighbors at each side were lower than expected. These results suggest that cockroaches

disperse through apartment walls horizontally. When there were three apartments adjoining each other by sharing a ceiling/floor, there was a trend that the observed frequencies for the three apartments being all uninfested or all infested were higher than expected, and the observed frequencies for an uninfested apartment having two infested neighbors or an infested apartment having two uninfested neighbors up and downstairs were lower than expected, suggesting that cockroaches disperse through apartment ceilings/floors vertically.

At 6 mo, the observed frequencies of infestations between apartments across the hallway and shared ceilings/floors were still significantly different from the expected. However, the observed frequency of infestations among apartments sharing walls was not significantly different from expected. Therefore, the infestations among apartments sharing walls became statistically independent at 6 mo.

At 12 mo, the observed frequencies were no longer significantly different from the expected except apartments sharing ceiling/floor. Infestations in apartments across the hallway and sharing walls were independent to each other. Therefore, implementation of building-wide cockroach IPM for a 12 mo period eliminated the correlations among infestations cross the hallway and sharing walls in the building. The results also suggested that infestations were correlated more strongly vertically, which may be caused by the structure of the building. The correlation of building structure and cockroach dispersal were described previously (Owens and Bennett 1982); Runstrom and Bennett (1990) found that most inter-apartment movement of cockroaches happened in adjacent kitchens, while in our case the correlation through ceiling/floor last for the longest time. Therefore, these cases may vary under different building structures.

The distributions of the new infestations found at 6 and 12 mo were statistically independent from the existing infestations at 0 and 6 mo, respectively. Therefore, building-wide IPM suppressed or did not promote the inter-apartment movement of cockroaches. It is reasonable to hypothesize that new infestations will be correlated to the existing infestations if there were no effective management taking place.

The 2 d and 14 d traps catches were positively correlated 3-5 wk after treatment. The linear regression indicates that for 2 d traps would fail to detect the infestations when the number of cockroaches is low (≤ 6). However, the slope was much smaller than 7 (since 14 d period is 7 times long as 2 d), indicating that the daily capture rate did not increase at a constant rate after treatment, which was most likely due to the decrease in the cockroach population overtime. The 2 d and 14 d trap catches were still positively correlated during 5-7 wk after treatment, with a smaller R^2 compared to the 3-5 wk period, indicating a weak linear correlation. The 2 d and 14 d trap catches 7-9 wk after treatment were not significantly correlated, possibly due to the impact of treatment on the cockroach population and smaller sample sizes.

After initial treatment, 2 d traps failed to detect as many as 30% infestations compared to 14 d traps. The failure rate tended to increase overtime and reached 39 and 48% during the 2nd and 3rd follow-ups respectively. This was likely due to the overall decrease in the cockroach population in treated apartments. Based on our results we found that the 2 d trap was not a reliable monitoring method for detecting low-level cockroach infestations, and the accuracy decreased overtime when the German cockroach populations were suppressed by the treatments.

In addition to increased sensitivity in detecting cockroaches, placing traps for 14 d would fit the treatment schedule better. For the conventional 1-7 d trap placement, researchers or pest management technicians need an extra trip to the field site after each follow-up treatment merely for trap pick-up (Miller and Meek 2004, Wang and Bennett 2006, Sever et al. 2007, Nalyanya et al. 2014). The 14 d trap placement allows applicators to combine follow-up treatment, and trap pick-up and replacement to the same day on a bi-weekly schedule: trap inspection can be done during the next visit for follow-up treatment.

In apartments which did not have cockroaches during both 0 and 6 mo inspections, extremely low cockroach activity (≈ 1 cockroach per trap during 6 mo) was detected in 11 of the 22 apartments. Therefore, cockroach introduction through dispersal among apartments is always present within a multi-unit building. Educating the residents even when they don't have cockroach issues should also be an important component of successful cockroach management programs. Proactive monitoring using traps placed for 14 d is also recommended for monitoring German cockroach infestations and evaluating program success.

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Table 1.1. Expected and observed frequency of three spatial infestation patterns at 0 mo (Infestation rate = 48.9%).

Type of spatial relationship	Infestation conditions	No.	Expected frequency (%)	Observed frequency (%)
Across the hallway*	Both uninfested	27	26.0†	30.7
	one infested	30	50.0	34.1
	Both infested	31	24.0	35.2
Sharing walls*	All uninfested	18	13.3	29.0
	Uninfested + one infested neighbor	14	25.5	22.6
	Uninfested + two infested neighbors	3	12.2	4.8
	Infested + two uninfested neighbors	2	12.7	3.2
	Infested + one uninfested neighbor	7	24.5	11.3
	All infested	18	11.8	29.0
Sharing ceiling/floor*	All uninfested	26	13.3	24.1
	Uninfested + one infested neighbor	33	25.5	21.5
	Uninfested + two infested neighbors	14	12.2	8.9
	Infested + two uninfested neighbors	10	12.7	6.3
	Infested + one uninfested neighbor	33	24.5	20.9
	All infested	31	11.8	18.4

†The total frequency within each type of spatial relationship may not be 100% due to rounding.

* Significant difference (Exact test of goodness-of-fit, $P < 0.05$).

Table 1.2. Expected and observed frequency of three spatial infestation patterns at 6 mo
(Infestation rate = 24.5%).

Spatial relationships	Infestation conditions	No.	Expected frequency (%)	Observed frequency (%)
Across the hallway*	Both uninfested	53	57.8	60.2
	one infested	23	36.5	28.4
	Both infested	10	5.8	11.4
Sharing walls	All uninfested	30	43.9	48.4
	Uninfested + one infested neighbor	18	27.7	29.0
	Uninfested + two infested neighbors	2	4.4	3.2
	Infested + two uninfested neighbors	3	13.9	4.8
	Infested + one uninfested neighbor	7	8.8	11.3
	All infested	2	1.4	3.2
Sharing ceiling/floor*	All uninfested	75	43.9	47.5
	Uninfested + one infested neighbor	37	27.7	23.4
	Uninfested + two infested neighbors	4	4.4	2.5
	Infested + two uninfested neighbors	16	13.9	10.1
	Infested + one uninfested neighbor	18	8.8	11.4
	All infested	8	1.4	5.1

* Significant difference (Exact test of goodness-of-fit, $P < 0.05$).

Table 1.3. Expected and observed frequency of three spatial infestation patterns at 12 mo
(Infestation rate = 12.2%).

Spatial relationships	Infestation conditions	No.	Expected frequency (%)	Observed frequency (%)
Across the hallway	Both uninfested	68	77.4	77.3
	one infested	19	21.1	21.6
	Both infested	1	1.4	1.1
Sharing walls	All uninfested	43	68.1	69.4
	Uninfested + one infested neighbor	10	18.6	16.1
	Uninfested + two infested neighbors	0	1.3	0.0
	Infested + two uninfested neighbors	5	9.3	8.1
	Infested + one uninfested neighbor	4	2.5	6.5
	All infested	0	0.2	0.0
Sharing ceiling/floor*	All uninfested	115	68.1	72.8
	Uninfested + one infested neighbor	23	18.6	14.6
	Uninfested + two infested neighbors	1	1.3	0.6
	Infested + two uninfested neighbors	10	9.3	6.3
	Infested + one uninfested neighbor	6	2.5	3.8
	All infested	3	0.2	1.9

* Significant difference (Exact test of goodness-of-fit, $P < 0.05$).

Table 2. The frequencies of whether new infestations at 6 and 12 mo had neighboring infestations 6 mo ago.

New infestations	Presence of neighboring infestations 6 mo ago	No.	Expected frequency (%)	Observed frequency (%)
6 mo	No neighboring infestation at 0 mo	3	35	20
	≥ 1 neighboring infestation (across hallway or sharing ceiling or walls) at 0 mo	12	65	80
12 mo	No neighboring infestation at 6 mo	9	45	60
	≥ 1 neighboring infestation (across hallway or sharing ceiling or walls) at 6 mo	6	55	40

Table 3. Cockroach detection using 2 d versus 14 d placement.

Time period after the initial treatment	Number of apartments with both 2 d and 14 d trap counts	No. of infestations based on 2 d placement	No. of infestations based on 14 d placement	Failure rate of 2 d traps comparing to 14 d traps (%)
3-5 wk	83	39	56	30
5-7 wk	58	27	44	39
7-9 wk	47	16	31	48

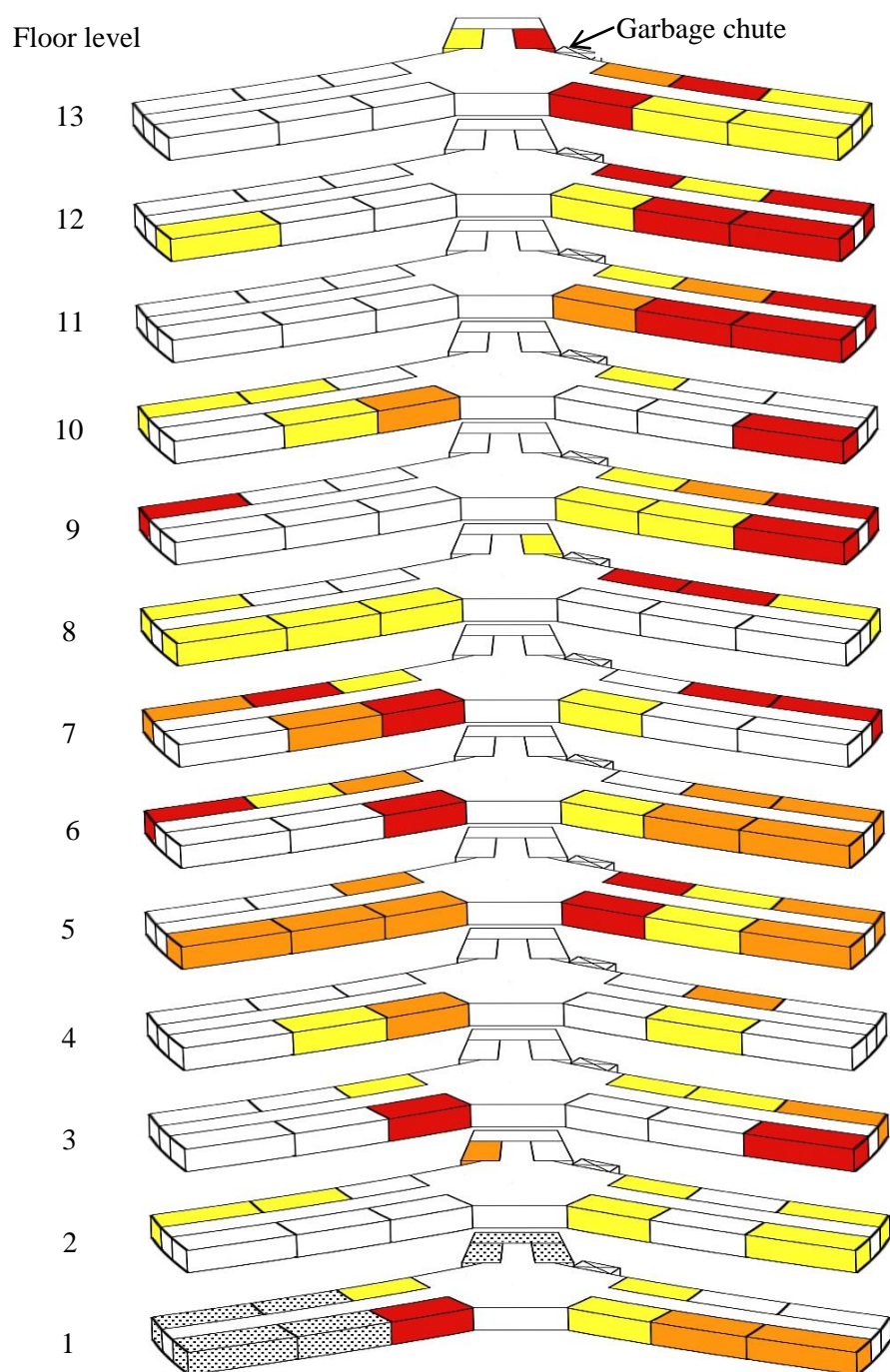


Fig. 1.1. Distribution of cockroach infestations in a 188-unit apartment building at 0 mo.

Each square represents one apartment. Squares filled with dots were non-residential units.

Different colors represent level of German cockroach population (Yellow- light

infestation (14 d trap catch 1-9); Orange- medium infestation (14 d trap catch 10-50);
Red- heavy infestation (14 d trap catch > 50)).

Floor level

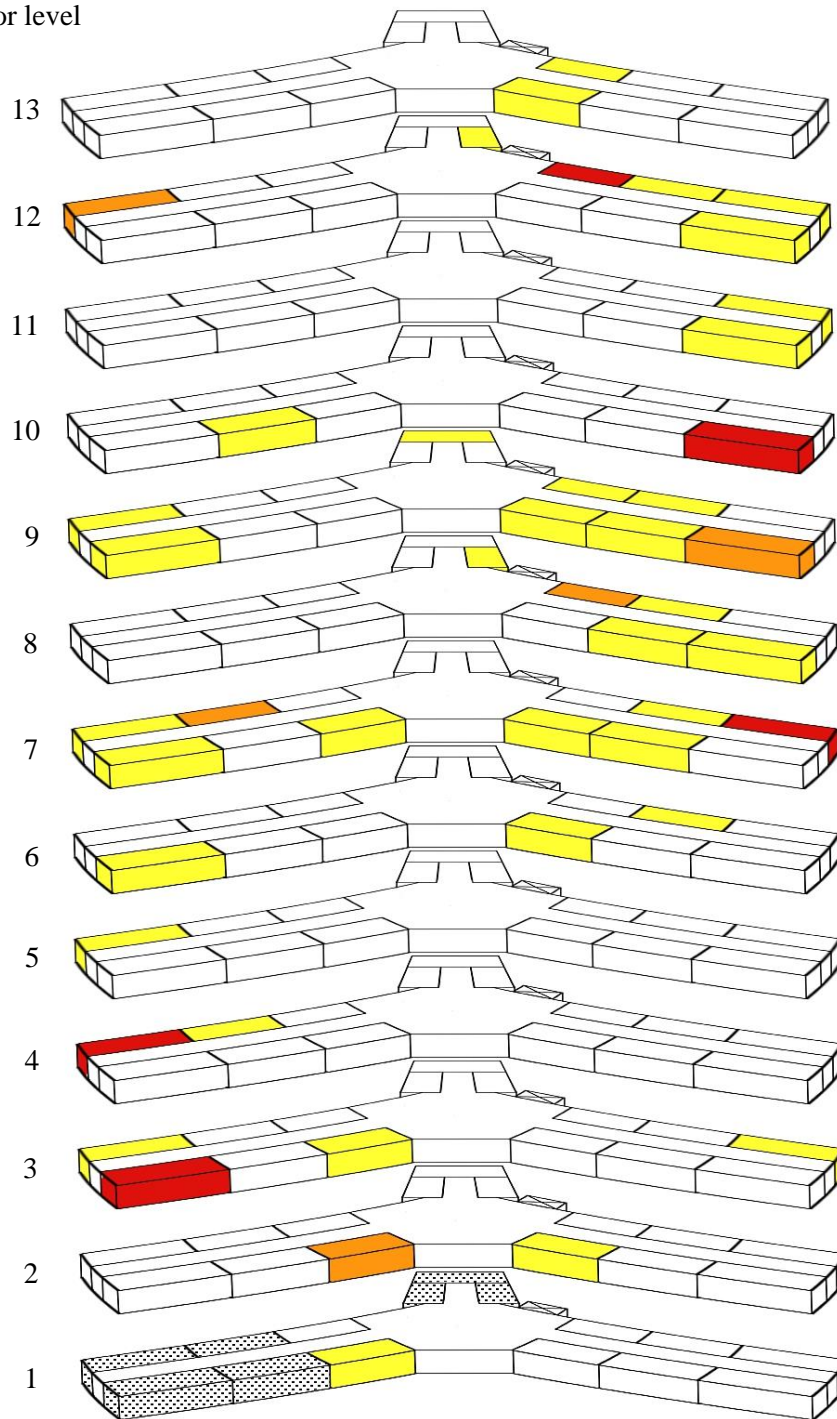


Fig. 1.2. Distribution of cockroach infestations at 6 mo.

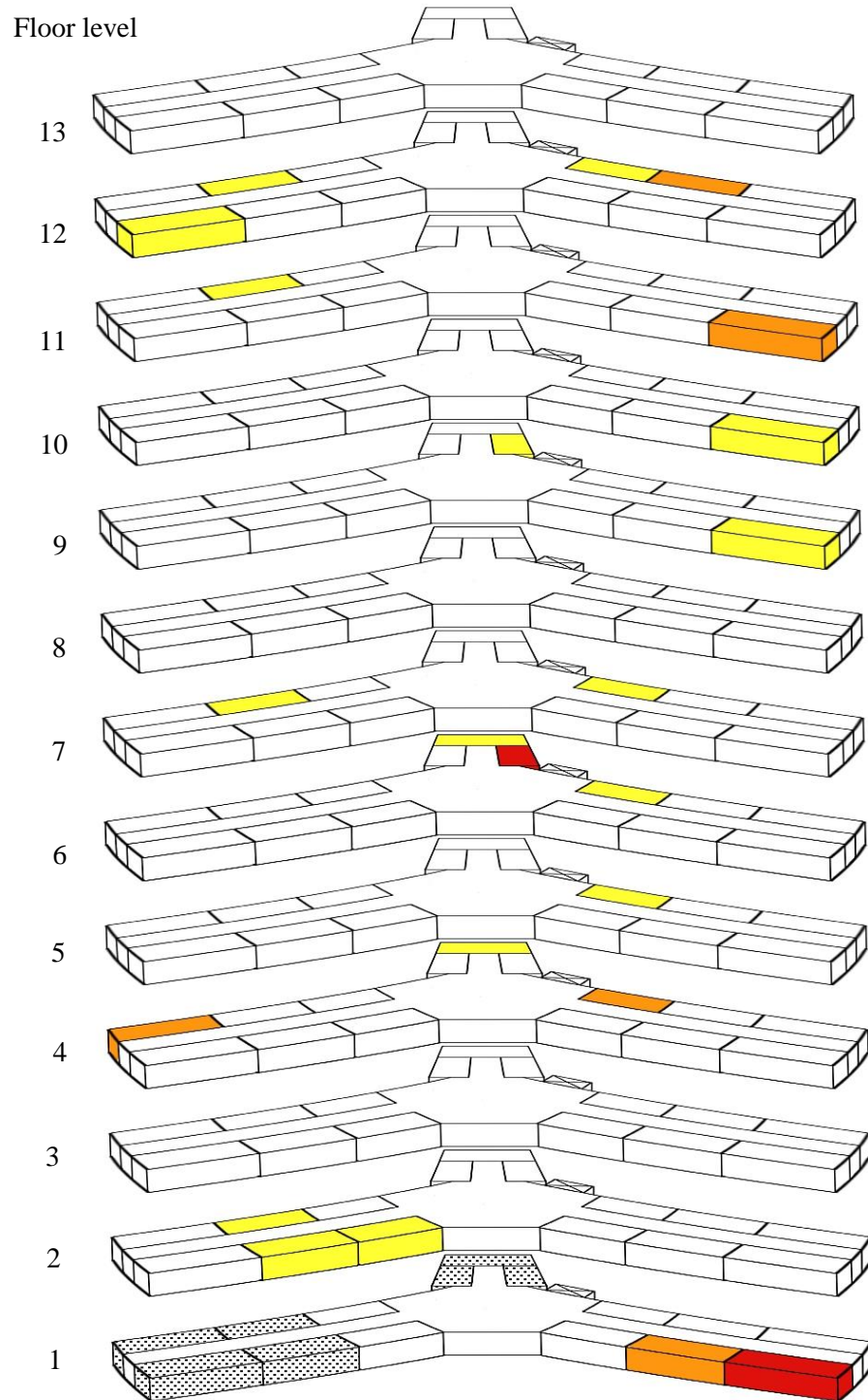


Fig. 1.3. Distribution of cockroach infestations at 12 mo.

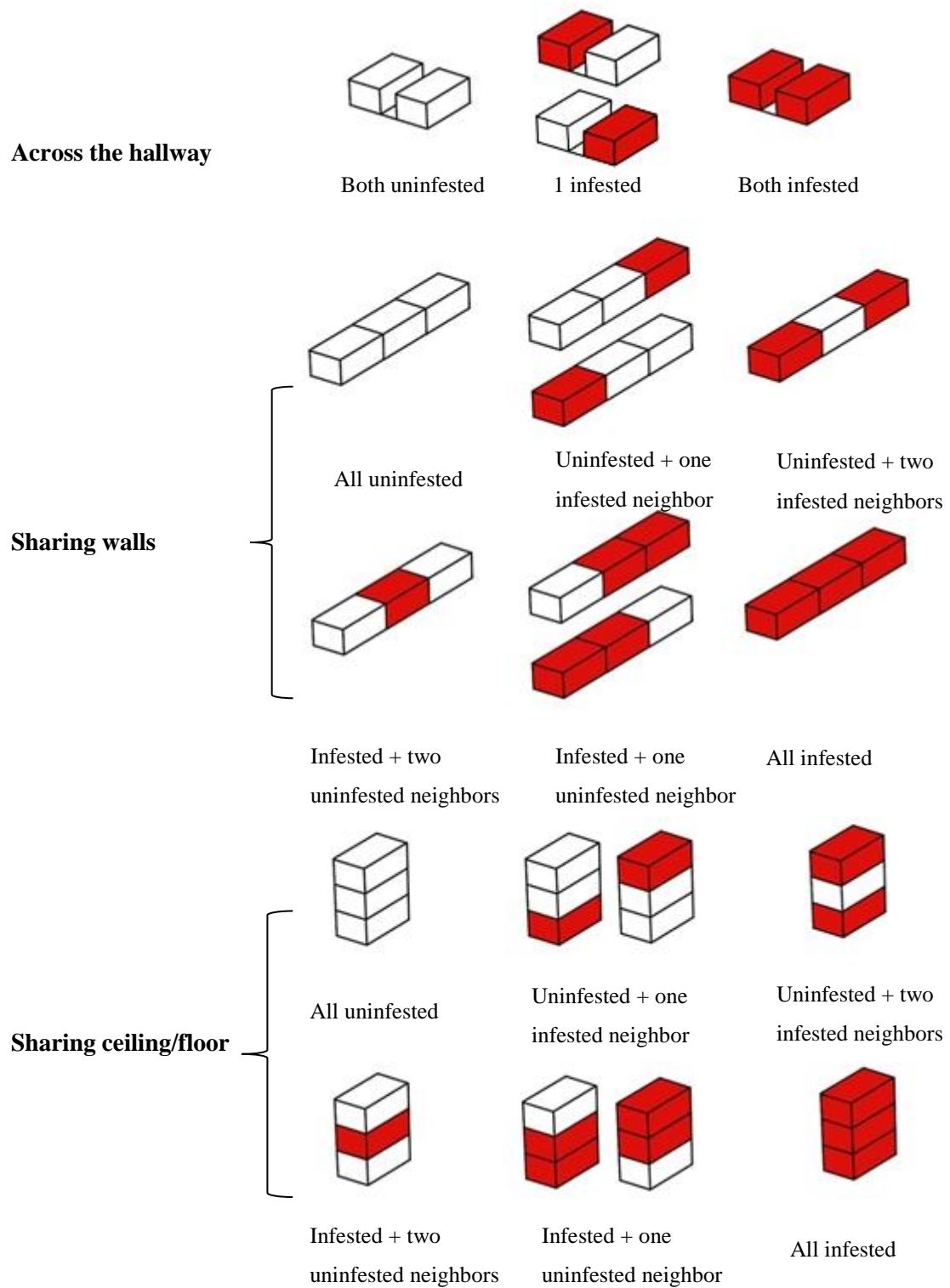


Fig. 2. Possible cockroach infestation patterns among units in the building. (Red- infested)

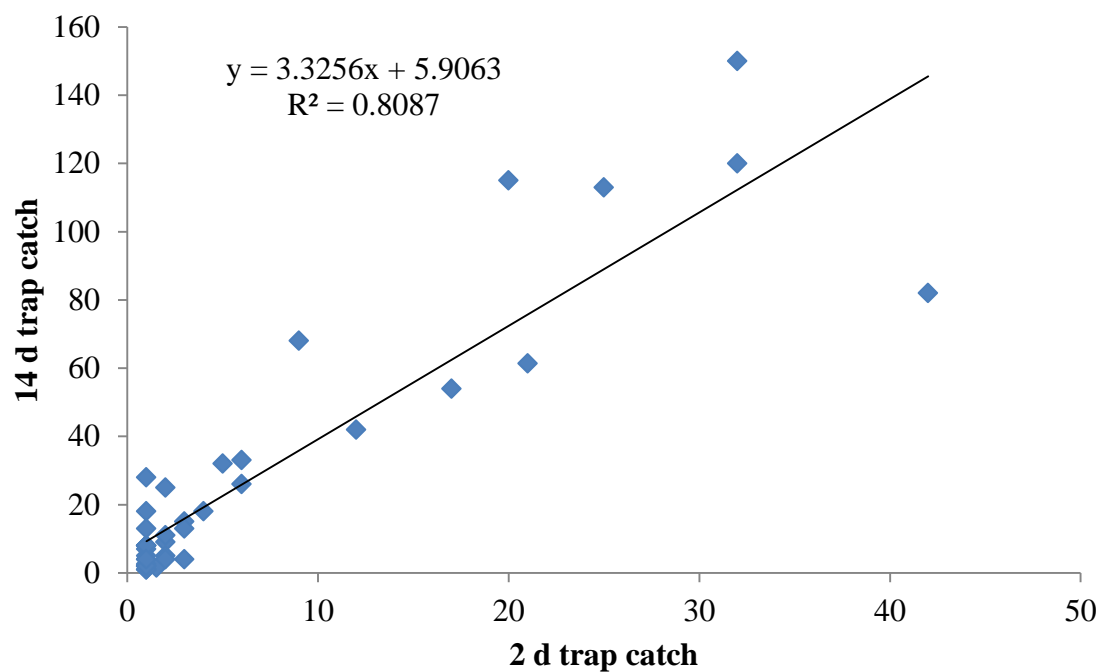


Fig. 3.1. Relationship between 2 d and 14 d trap catch in cockroach infested apartments during 3-5 wk after treatment (n = 39).

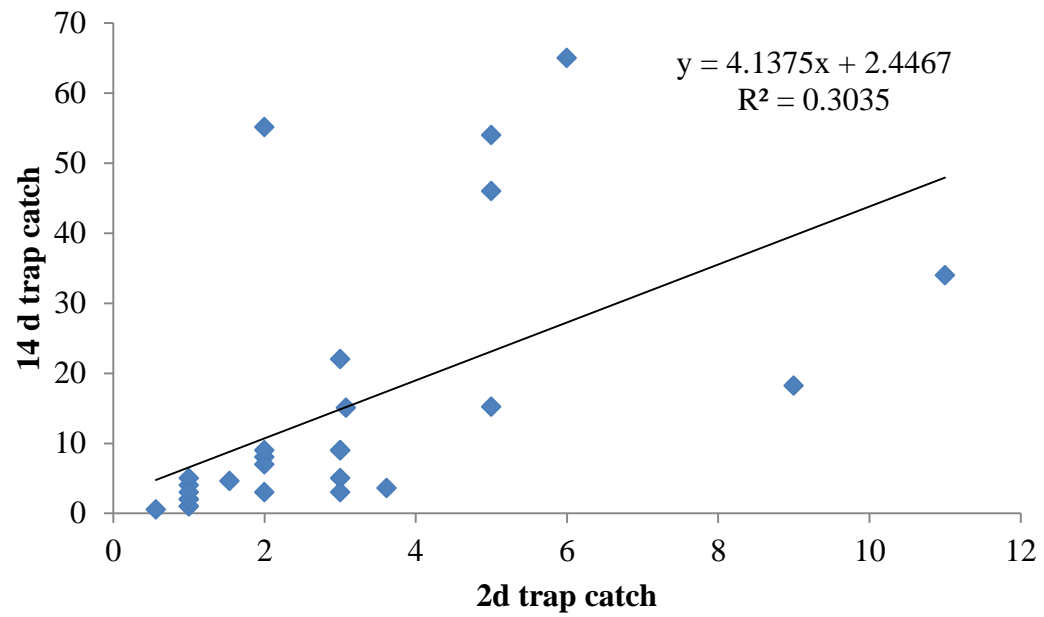


Fig. 3.2. Relationship between 2 d and 14 d trap catch in cockroach infested apartments during 5-7 wk after treatment (n = 27).

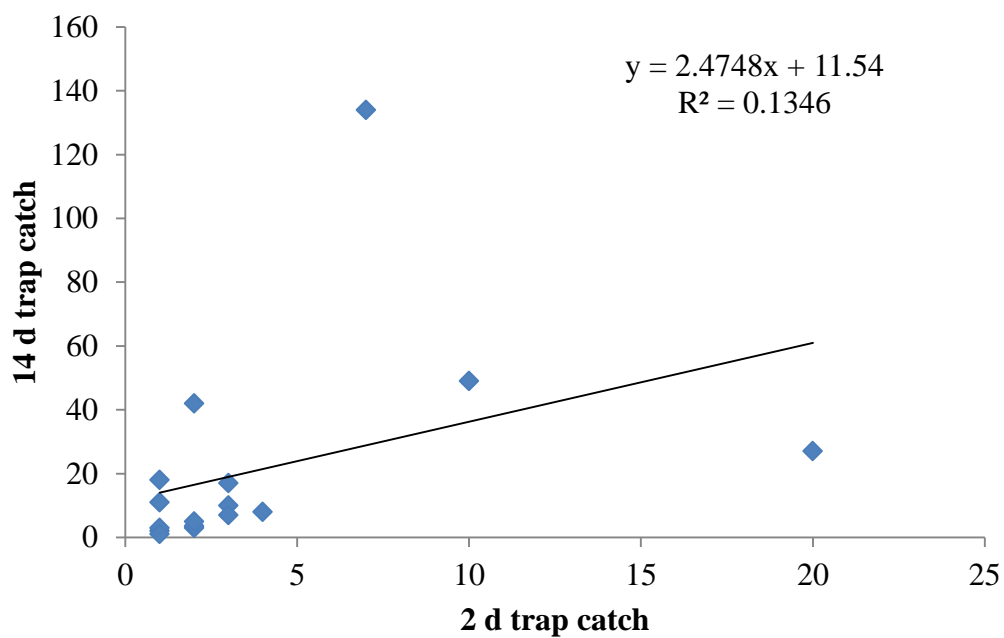


Fig. 3.3. Relationship between 2 d and 14 d trap catch in cockroach infested apartments during 7-9 wk after treatment (n = 16).

CHAPTER THREE

Toxicities of Selected Essential Oils, Silicone Oils, and Paraffin Oil against the Common Bed Bug, *Cimex lectularius* L. (Hemiptera: Cimicidae)

Abstract

The common bed bug (*Cimex lectularius* L.) resurged in the U.S. and many other countries over the past decade. The need for safe and effective bed bug control products propelled the development of numerous “green pesticides”, mostly with essential oils listed as active ingredients. Various inorganic and organic oils also were used for bed bug management. However, there are no published studies on their toxicities against bed bugs except for evaluations of essential-oil based insecticide products. In this study, we screened 18 essential oils, 3 silicone oils, and paraffin oil for their toxicities against bed bugs. All the oils exhibited insecticidal activity in topical assays. Their toxicities varied significantly; all of the evaluated essential oils were less effective than silicone oils and paraffin oil. The LD₅₀ values of the most effective essential oil (blood orange), paraffin oil (C5-20 Paraffins), and the most effective silicone oil Dodecamethylpentasiloxane) are 0.184 ± 0.018 , 0.069 ± 0.012 , and 0.036 ± 0.005 mg/bug, respectively. Direct spray of 1% water solution of 3 - [Hydroxy (polyethyleneoxy) propyl] heptamethyltrisiloxane, the only silicone oil in our study which mixes well with water, resulted in 92% bed bug mortality after 1 d. Results of this study indicate silicone oils and paraffin oil have the potential to be used as safer alternative bed bug control materials.

Introduction

The common bed bug, *Cimex lectularius* L. (Hemiptera: Cimicidae), is an obligate blood feeder, which resurged world-wide in recent decades (Doggett et al. 2004,

Davies et al. 2012). Insecticide treatment is frequently used by professionals as well as residents to eliminate bed bug infestations. In a senior citizen occupied building in Indiana, 40% of the 40 interviewed residents indicated they self-applied pesticides for bed bug control (Wang et al. 2010), while 89% of 18 interviewed residents treated bed bugs on their own in another study in New Jersey (Wang et al. 2014). Professional products such as Temprid SC (21% imidacloprid + 10.5% β -cyfluthrin; Bayer Environmental Science, Research Triangle Park, NC), Suspend SC (4.75% deltamethrin; Bayer Crop Science, Research Triangle Park, NC), and Demand CS (9.7% λ -cyhalothrin ; Zeneca Inc., Wilmington, DE) are widely utilized by licensed pesticide applicators in the U.S.

Compared to professionals, fewer control options exist for residents. The majority of synthetic consumer bed bug control insecticides on the U.S. market are pyrethrins or pyrethroids, with a few exceptions like silica gel and diatomaceous earth dusts (Wang et al. 2014). The effectiveness of pyrethrins and pyrethroids against bed bugs is questionable, due to the wide-spread resistance to pyrethroids among bed bug populations (Romero et al. 2007, Zhu et al. 2010, Davies et al. 2012). Besides pyrethrin and pyrethroids, many essential oil-based products for controlling bed bugs have been developed in recent years. One of the reasons companies develop product lines with essential oils as “green pesticides” is that many products are exempt from the requirements of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) under the minimum risk exemption regulations (U.S. EPA 2015); therefore, manufacturers do not need to pay fees for U.S. EPA registration and they can get their product to market quicker, and are exempt from EPA regulation. As EPA exempt products, efficacy testing

is not required, and many of these products are ineffective. Out of 11 tested essential oil-based pesticides and detergents, only EcoRaider[®] bed bug killer (1% geraniol + 1% cedarwood oil + 2% sodium lauryl sulfate; Reneotech, Inc., North Bergen, NJ) and Bed Bug Patrol (0.003% clove oil + 1% peppermint oil + 1.3% sodium lauryl sulfate; Nature's Innovation Inc., Buford, GA) caused > 90% mortality to bed bug nymphs when using direct spray evaluation (Singh et al. 2014). In a field study, EcoRaider caused similar bed bug population reduction as Temprid SC and EcoRaider + Temprid SC mixture spray (Wang et al. 2014). The different efficacies among the essential oil based products indicate the necessity for further investigation of the relative toxicity of individual essential oils.

Numerous essential oils are shown to be effective against insect pests in laboratory and field assays and are used for pest control. For example, neem oil has been used for decades to control pests such as brown planthopper (*Nilaparvata lugens* Stål), black bug (*Scotinophara lurida* Burm.), earhead bug (*Leptocorisa acuta* Thunb.), as well as fungus like sheath rot (*Sarocladium oryzae* Saw.) (Isman et al. 1990, Schmutterer 1990, Lokanadhan et al. 2012). Thyme and peppermint are used to control greenhouse whiteflies (*Trialeurodes vaporariorum* Westw.) (Aroiee et al. 2005). Eucalyptus oil is effective against insects and other organisms such as bacteria, fungi, nematodes, mites, and weeds (Batish et al. 2008). Essential oils also have potential in structural and household pest management. Polyethylene glycol coated nanoparticles loaded with garlic essential oil is effective against red flour beetle (*Tribolium castaneum* Herbst) (Yang et al. 2009). Phillips et al. (2010) evaluated the toxicities of 12 essential oils against German

cockroaches (*Blattella germanica* L.), and found thymol and trans-cinnamaldehyde to be the most effective.

Many silicone oils are stable and safe chemical materials, commonly used in many daily necessities and medications that make direct contact to the human body, and sometimes even used during surgeries. Silicone oil can be used as a lubricant (Yoo and Kim 2013), in shampoo (Reid and Murray 1992), medicine (Sawada and Sone 1990), and other products used in daily life. Silicone oil is used in coating surfaces of various medical equipment (e.g., syringes and stoppers) to facilitate processing or to inhibit protein binding (Jones et al. 2005). Silicone oil injections are common in vitreous microsurgery (Cox et al. 1986). Interestingly, they have some properties that also make them an excellent candidate for pesticides. These oils are generally considered as unreactive and safe to humans. Many silicone oils are light in weight, odorless, and colorless, which make them easy to apply with little issue of clogging, staining, and smelling. Some silicone oils are water soluble. A silicone oil, Silwet L-77, is a non-ionic surfactant, and has been proven to be effective against multiple agricultural pests (Imai et al. 1995, Purcell and Schroeder 1996, Tipping et al. 2003). A few other trisiloxane surfactants have insecticidal activity against two spotted spider mite (Cowles et al. 2000). Despite the fact that silicone oils themselves may have insecticidal activities, they are more often listed as inert ingredients in pesticide formulations (Knoche 1994). The toxicities of silicone oils against bed bugs have not been studied previously.

Paraffin oil, or liquid paraffin, is a transparent, colorless, and odorless mineral oil. Similar to silicone oil, paraffin oil is safe to humans and frequently used in commonly used products, such as lamp oil and medications. Liquid paraffin is often used as stool

lubricant for treating constipation and encopresis (Sharif et al. 2001). The use of mineral oil in pest management has been tested in previous research. When mixed with synthetic pesticides, Luxan Oil H (93% mineral oil), inhibited the spread of aphid transmitted lily symptomless virus and lily mottle virus in *Lilium* L. (Liliales: Liliaceae) (Asjes and Blom-Barnhoorn 2000). When applied alone, mineral oil provided additional control of codling moth and secondary pests on apple, although the benefit was hard to detect when the pest density was high (Fernandez et al. 2005). Sunspray oil, a mineral oil, showed repellency to silver leaf whitefly (*Bemisia argentifolii* Bellows & Perring) when applied on melon leaves under lab conditions (Liang and Liu 2002). Whether mineral oils could also be used in bed bug control has not been studied. Paraffin oil was also chosen in our study to evaluate toxicity against bed bugs because it is a light oil and will have less likelihood to stain mattresses, furniture, and other personal belongings compared to heavier oils.

In this study, 22 different essential oils, three silicone oils, and one paraffin oil were tested in the laboratory for their toxicities against bed bugs. Among the essential oils chosen for the study, cedarwood and palmarosa (geraniol) are active ingredients of EcoRaider; five different cedarwood oils were included because the species of cedarwood for extracting the oil used in EcoRaider is undisclosed by the manufacturer. The other essential oils are also readily available and have shown toxicities against insects in previous studies, as listed in Table 1. Two of the three silicone oils are listed as inert ingredients in a bed bug control product (BestYet, CedarCide Industries, Inc., Spring, TX), and the other is listed in a formula under development (Siltech Corporation, Inc., Toronto, Ontario, Canada). These silicone oils exhibited high toxicities against bed bugs in our

preliminary studies. Our goal is to identify safe and effective alternative chemicals for controlling bed bugs.

Materials and Methods

Chemicals. A total of 18 essential oils, three silicone oils, and paraffin oil were included in the study (Table 1). An essential oil-based consumer product, EcoRaider, was also used in this study to compare against pure essential oils.

Insects. A field strain of bed bug (Indy strain) was used in this study. It was collected from multiple apartments during 2008-2009 in a building in Indiana, and was moderately resistant to pyrethroids (Singh et al. 2014). Bed bugs were maintained in plastic containers (5 cm diameter and 4.7 cm height; Consolidated plastics, Stow, OH) with folded construction paper (40 mm length and 30 mm width; Universal Stationers Supply Co., Deerfield, IL) as harborages and held at $26 \pm 1^\circ\text{C}$, $40 \pm 10\%$ relative humidity (RH), and a 12:12 h L:D photoperiod. They were fed biweekly on defibrinated rabbit blood (Hemostat Laboratories, Dixon, CA) using a Hemotek membrane-feeding system (Discovery Workshops, Accrington, UK). Bed bugs were fed 1 week prior to all experiments.

Experiment 1. Initial screening of 22 oils. A total of 18 essential oils, 3 silicone oils, and paraffin oil were screened for their toxicities through topical assay. All oils were diluted to 0.2 g/ml in acetone. Adult male bed bugs of unknown age were used in this experiment. A droplet of 0.5 μl liquid was delivered onto each bed bug's dorsal thorax using a hand micro-applicator (Burkard Manufacturing Co. Ltd., Rickmansworth, Hertfordshire, UK), which delivered approximately 0.1 mg oil per bug. This dosage was chosen after preliminary tests. Bed bugs were treated in groups of 10 on Fisherbrand® P5

filter paper (3.5 cm diameter, Fisher Scientific, Pittsburgh, PA) in a petri dish (5.5 cm diameter and 1.5 cm height, Fisher Scientific, Pittsburgh, PA). Each treatment was replicated 4 times. Bed bugs in the control group were treated with acetone with the same number of bugs and replications.

The treated bed bugs were kept in an incubator (Percival Scientific, Inc., Perry, IA) at 25°C, a 12:12 h D:L light cycle, and were observed at 1, 3, 5, 7, and 14 d post-treatment. The numbers of dead and moribund bed bugs was recorded at each observation period. A dead bug was defined as no signs of activity when gently prodded with a pair of lightweight forceps. A moribund bug was defined as bug that could still move its' appendages, when prodded, but could not turn over or walk. Both dead and moribund bugs were included in mortality during analysis.

After the initial screening, the three silicone oils were tested again using the same method at a lower dose (0.04 mg oil per bug) to determine if there are significant differences in toxicities among them, since the toxicities of silicone oils were not separated by the dose of 0.1 mg oil per bug.

Experiment 2. Evaluating LD₅₀ of selected oils. Three oils (blood orange, paraffin oil, silicone oil #1) were included in this experiment. Blood orange and silicone oil #1 were the most effective oils in the respective category based on the results of Experiment 1. Adult males and females of mixed ages were used in this experiment. Tested solutions were applied to bed bugs using the same method as in Experiment 1. Blood orange was diluted to 0.2, 0.3, 0.4, 0.5, and 0.6 g/ml, to deliver doses of 0.1, 0.15, 0.2, 0.25, and 0.3 mg oil per bug, respectively. Paraffin oil and silicone oil #1 was diluted to 0.04, 0.08, 0.12, 0.16, and 0.2 g/ml, to deliver doses of 0.02, 0.04, 0.06, 0.08, and 0.1

mg oil per bug, respectively. The dose ranges for different oils were determined by preliminary tests. Eight bed bugs (4 males and 4 females) were used in each treatment and each treatment had 4 replications (N=32 bed bugs per treatment). Bed bugs in the control group were treated with acetone.

After treatment, the bed bugs were maintained in the same manner as in Experiment 1. The numbers of dead and moribund bed bugs were recorded at 1, 3, and 14 d post-treatment. Both dead and moribund bugs were included in mortality during analysis, and mortality at 3 d was used to calculate LD₅₀.

Experiment 3. Comparison of different cedarwood oils, geraniol, and EcoRaider by topical assay. This experiment was designed to investigate if the origin and source plants affects the efficacy of essential oils and to compare the efficacy of formulated essential oils (EcoRaider) and individual essential oil components. EcoRaider, five cedarwood oils, and geraniol were included in this experiment. The oils represented the two major active ingredients in EcoRaider: cedarwood oil and geraniol. The five cedarwood oils were from different source plants and origins, and have different chemical components (Table 1). The type of cedarwood oil used in EcoRaider was not disclosed by the manufacturer. A droplet of 0.5 µl EcoRaider (original product) or essential oils (0.2 g/ml in acetone) were applied onto bed bugs through topical assay, following the same method described in Experiment 1. Bed bug mortalities were recorded at 1, 3, 5, and 7 d post-treatment. Bed bugs in the control group were treated with acetone. After treatment, the bed bugs were held in the same way as in Experiment 1.

Experiment 4. Efficacy of silicone oil #3 water solution against bed bugs by direct spray. Silicone oil #3 was the only tested silicone oil which can be evenly mixed

with water without surfactant, which could be an advantage in formulation development. Bed bugs (10 males and 10 3rd-5th instar nymphs) were placed in a petri dish (5.5 cm diameter and 1.5 cm height) lined with filter paper (3.5 cm diameter) on the bottom and sprayed with 0.1, 0.5 and 1% silicone oil #3 water solutions. 6 ml solutions were applied to insects using a Potter spray tower (Burkard Scientific Ltd, Herts, UK) at the rate of 4.07 mg/cm² (1 gallon/1,000 ft²). Each treatment was replicated 3 times, and water was used in the control (N=60 bed bugs per treatment). The bed bugs were held in the same manner following treatment, as in Experiment 1. Mortality of bed bugs was observed at 1 d post-treatment.

Statistical analysis. The efficacies of different oils in Experiment 1 were compared and ranked by Kruskal-Wallis test because data of some tested groups were not normally distributed even after transformation (Shapiro–Wilk test). Probit analysis was used to calculate LD₅₀ values in Experiment 2. The mortalities caused by different cedarwood oils and geraniol in Experiment 3 were compared by repeated measures ANOVA followed by Tukey's HSD test. All mortalities were not adjusted by Abbott's formula because the mortalities in all controls were 0%. All analyses were performed using SAS software version 9.3 (SAS Institute Inc. 2011).

Results

Toxicities of 22 oils against bed bugs. Bed bug mortalities stabilized at 3 d after topical application of selected oils; therefore 3 d data were used for comparison. There were significant differences in mean bed bug mortalities ($\chi^2 = 62.67$, df = 21, $P < 0.001$), varying from $5 \pm 3\%$ (spearmint) to $100 \pm 0\%$ (silicone oil #1) (Fig. 1). The three silicone oils and the paraffin oil had highest mean rank scores among all the oils, and blood

orange had the highest mean rank score among all essential oils (Kruskal-Wallis test) (Table 2). There was no mortality in the control.

At the lower dose of 0.04 mg/bug, the silicone oil #1, #2, and #3 caused $65 \pm 3\%$, $25 \pm 7\%$, and $33 \pm 3\%$ mortality respectively; the efficacies of the three silicone oils were statistically different ($\chi^2 = 8.10$, $df = 2$, $P = 0.017$), with a mean rank score of 10.5 for silicone oil #1, 3.8 for silicone oil #2, and 5.3 for silicone oil #3 (Kruskal-Wallis test). There was no mortality in the control.

LD₅₀ of selected oils. Because a few bugs that were knocked down at 1 d recovered at 3 d, only mortality data at 3 d were used in LD₅₀ value analysis. Dead or moribund bugs on day 3 did not recovered after day 14. The LD₅₀ values of blood orange, paraffin oil, and silicone oil #1 were 0.184 ± 0.018 , 0.069 ± 0.012 , and 0.038 ± 0.005 mg/bug, respectively (Table 3). The relative toxicities of these three oils were silicone oil #1 > paraffin oil > blood orange. There was no mortality in the control.

Comparison of different cedarwood oils, geraniol, and EcoRaider by topical assay. The mortalities caused by different treatments were significantly different ($F = 5.27$; $df = 6, 21$; $P = 0.002$); time after treatment (1, 3, 5, and 7 d) also affected mortalities significantly ($F = 13.53$; $df = 3, 63$; $P < 0.001$). There was an interaction between treatment and time ($F = 11.74$; $df = 18, 63$; $P < 0.001$). From 1 d to 7 d, the mortalities for different essential oil treatments did not change significantly overtime (cedarwood Atlas: $t = -0.47$; $df = 57$; $P = 0.643$. cedarwood Chinese: $t = 0.47$; $df = 57$; $P = 0.643$. cedarwood Himalayan: $t = -0.93$; $df = 57$; $P = 0.355$. cedarwood Texas: $t = 0.47$; $df = 57$; $P = 0.643$. cedarwood Virginian: $t = -0.47$; $df = 57$; $P = 0.643$. geraniol: $t = -0.47$; $df = 57$; $P = 0.643$). However, the mortality in the EcoRaider treatment increased

significantly from $38 \pm 10\%$ to $93 \pm 5\%$ ($t = -10.27$; $df = 57$; $P < 0.001$) (Fig. 2). The mortality in the EcoRaider treatment was significantly higher than other treatments at 7 d (Tukey's HSD test, $P = 0,05$). There was no mortality in the control.

Efficacy of silicone oil #3 water solution against bed bugs by direct spray. The silicone oil #3 solution killed 15 ± 5 , 88 ± 2 , and $92 \pm 5\%$ of the bed bugs treated at the concentrations of 0.1, 0.5, and 1% after 1 d in direct spray (6 ml solution applied at the rate of 4.07 mg/cm^2), respectively. The mortalities after 7 d were 18 ± 5 , 90 ± 0 , and $92 \pm 5\%$ at the concentrations of 0.1, 0.5, and 1%, respectively. There was no mortality in the control.

Discussion

All the tested oils showed some level of toxicity against bed bugs. However, the toxicities of different oils varied according to oil type, origin, and source plants. For example, there are different types of cedarwood oils. The five cedarwood oils included in our study were from four origins, extracted from six different tree species, and had different main constituents. Their toxicities varied significantly. Therefore, it is necessary to understand their differences and clearly list the sources when used for pest management purposes.

Our study revealed that paraffin oil and the three silicone oils were ranked higher in toxicity than all the essential oils tested. Besides higher toxicities, paraffin oil and silicone oils have additional advantages over essential oils as insecticides. Paraffin and silicone oils are generally odorless and colorless, while essential oils usually have strong odors (Pol et al. 2006, Koliou et al. 2010). Although the odors from essential oils are often described as fragrant and pleasing, to some individuals they can be unpleasant and

even irritating. Some essential oils such as blood orange, are colored and may stain treated items. Paraffin oil and silicone oils hold promise as safe and effective alternative bed bug control materials.

Little is known about the modes of action of silicone oils and paraffin oil against insects. Tipping et al. (2003) suggested that the toxicity of certain silicone oils were likely due to their superior wetting properties, which disrupt a variety of arthropod systems that depend on specific water relationships including cuticular and respiratory functions. This hypothesis may apply to paraffin oil as well. Further examination by microscopic tools such as scanning electron microscope on bugs treated with silicone and paraffin oils may help us understand the modes of action of these oils.

The different time-mortality patterns of EcoRaider compared with individual essential oils suggest that by combining different essential oils or using special formulating techniques, the efficacy of essential oils may be significantly increased. In Experiment 3, comparing different oils and EcoRaider, the total amount of active ingredients applied to each insect was much higher in the cedarwood oil and geraniol treatments (0.2 g/ml in acetone) compared to EcoRaider (1% geraniol + 1% cedarwood oil). Yet, EcoRaider resulted in much higher mortality. The additional mortality after 7 d treatment in the EcoRaider treatment also suggests that formulated essential oils could have additional insecticidal effect beyond the initial kill shortly after exposure. The additional slow killing effect and high efficacy are probably a result of the interactions of multiple ingredients and a special formulating process. For example, one possibility is that the surfactants in EcoRaider allow continuous penetration of essential oil components through insect cuticle. The existence of synergistic effects needs further tests

by comparing the efficacy of single and combined ingredients in solutions; and future studies are warranted for developing more effective formulations based on various oils.

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Table 1. Essential oils, silicone oils, and paraffin oil tested in Experiment 1.

Oil	Source plant	Country of origin	Main constituents and reference	Manufacturer and registration number
Anise star	<i>Illicium verum</i>	China	82.5% Anethole (Chang and Ahn 2002)	New Direction Aromatics Inc. (Mississauga, ON, Canada). CAS# 8007-70-3
Basil Sweet	<i>Ocimum basilicum</i> , <i>ct. Estragole</i>	India	73.06% Methyl chavicol (estragole); 19.2% Linalool (Chang et al. 2009)	New Direction Aromatics Inc. (Mississauga, ON, Canada). CAS# 8015-73-4
Blood orange	<i>Citrus sinensis</i>	Italy	98% Limonene (Hollingsworth 2005)	New Direction Aromatics Inc. (Mississauga, ON, Canada). CAS# 8028-48-6
Cedarwood Atlas	<i>Cedrus atlantica</i>	Morocco	45.95% b-Himalachene ¹	New Direction Aromatics Inc. (Mississauga, ON, Canada). CAS# 8000-27-9
Cedarwood Chinese	<i>Cupressus funebris</i>	China	14.75% Thujopsene; 10.19% a-Cidrene; 10% Cedrol	New Direction Aromatics Inc. (Mississauga, ON, Canada). CAS# 8000-27-9
Cedarwood Himalayan	<i>Cedrus deodora</i>	India	50.04% Himalchene	New Direction Aromatics Inc. (Mississauga, ON, Canada). CAS# 68991-36-6
Cedarwood Texas	<i>Juniperus ashei</i> / <i>mexicana</i>	USA	34% Thujopsenene; 26% Cedrol	New Direction Aromatics Inc. (Mississauga, ON, Canada). CAS# 68990-83-0
Cedarwood Virginian	<i>Juniperus virginiana</i>	USA	22.53% Cedrol; 20.31% Thujopsenene	New Direction Aromatics Inc. (Mississauga, ON, Canada). CAS# 8000-27-9
Eucalyptus Blue Mallee	<i>Eucalyptus polybractea</i>	Australia	87.40% 1,8-Cineole (Lee et al. 2004)	New Direction Aromatics Inc. (Mississauga, ON, Canada). CAS# 8000-48-4
Eucalyptus Dives	<i>Eucalyptus dives</i>	Australia	52.07% Piperitone (Abdelgaleil et al. 2008); 20.38% a-phellandrene	New Direction Aromatics Inc. (Mississauga, ON, Canada) CAS# 90028-48-1
Eucalyptus Lemon	<i>Eucalyptus citriadora</i>	China	82.1% Citronellal (Koul et al. 2008)	New Direction Aromatics Inc. (Mississauga, ON, Canada) CAS# 85203-56-1
Neem	<i>Azadirachta</i>	-	Azadirachtin (Isman et al.	Lotus Brands, Inc. (Twin Lakes, WI)

	<i>indica</i>		1990)	
Oregano	<i>Origanum vulgare</i>	Hungary	Carvacrol, thymol (Lambert et al. 2001)	Puritan's Pride, Inc. (Oakdale, NY)
Palmarosa	<i>Cymbopogon martinii var motia</i>	India	83.8% Geraniol	New Direction Aromatics Inc. (Mississauga, ON, Canada). CAS# 8014-19-5
Peppermint	<i>Mentha × piperita</i> L.	India	Menthol, menthone (Edris and Farrag 2003)	Puritan's Pride, Inc. (Oakdale, NY)
Spearmint	<i>Mentha spicata</i>	China	L-carvone (Koul et al. 2008)	New Direction Aromatics Inc. (Mississauga, ON, Canada)
Tea Tree	<i>Melaleuca alternifolia</i>	China	41.02% Terpinen-4-ol (Isman 2000)	New Direction Aromatics Inc. (Mississauga, ON, Canada). CAS# 68647-73-4
Thyme	<i>Thymus vulgaris</i>	-	Thymol (Juven et al. 1994)	Envisage Essentials (Canton, GA)
Silicone oil #1	-	-	100% Dodecamethylpentasiloxane	Clearco Products Co., Inc. (Bensalem, PA). CAS# 141-63-9
Silicone oil #2	-	-	100% Cyclopentasiloxane	Clearco Products Co., Inc. (Bensalem, PA). CAS# 541-02-6
Silicone oil #3	-	-	90% 3-[Hydroxy (polyethyleneoxy) propyl] heptamethyltrisiloxane	Gelest, Inc. (Morrisville, PA). CAS# 67674-67-3
Paraffin oil	-	-	100% C5-20 Paraffins	Lamplight Farms Inc. (Menomonee, WI). CAS# 64771-72-8

Table 2. Mean rank scores of the toxicities of different oils ranked by Kruskal-Wallis Test.

Oil	Mean rank score	Oil	Mean rank score
Silicone oil #1	83.0	Tea Tree	40.3
Silicone oil #3	81.0	Eucalyptus Blue Mallee	36.1
Silicone oil #2	79.0	Cedarwood Texas	35.5
Paraffin oil	79.0	Cedarwood Atlas	32.8
Blood orange	66.0	Palmarosa	32.8
Cedarwood Chinese	57.0	Peppermint	27.1
Cedarwood Virginian	51.6	Eucalyptus Dives	26.5
Thyme	51.6	Neem	23.0
Oregano	44.8	Basil Sweet	20.8
Cedarwood Himalayan	43.8	Anise star	15.1
Eucalyptus Lemon	40.6	Spearmint	11.8

Table 3. Probit analysis results of the tested oils.

Oil	n	Slope \pm SE	LD₅₀ (95% CI) (mg/bug)	χ^2	P
Blood orange	160	9.71 \pm 1.51	0.184 (0.166-0.202)	41.48	< 0.001
Paraffin oil	160	6.15 \pm 1.12	0.069 (0.060-0.081)	30.25	< 0.001
Silicone oil #1	160	7.66 \pm 1.15	0.038 (0.032-0.043)	44.29	< 0.001

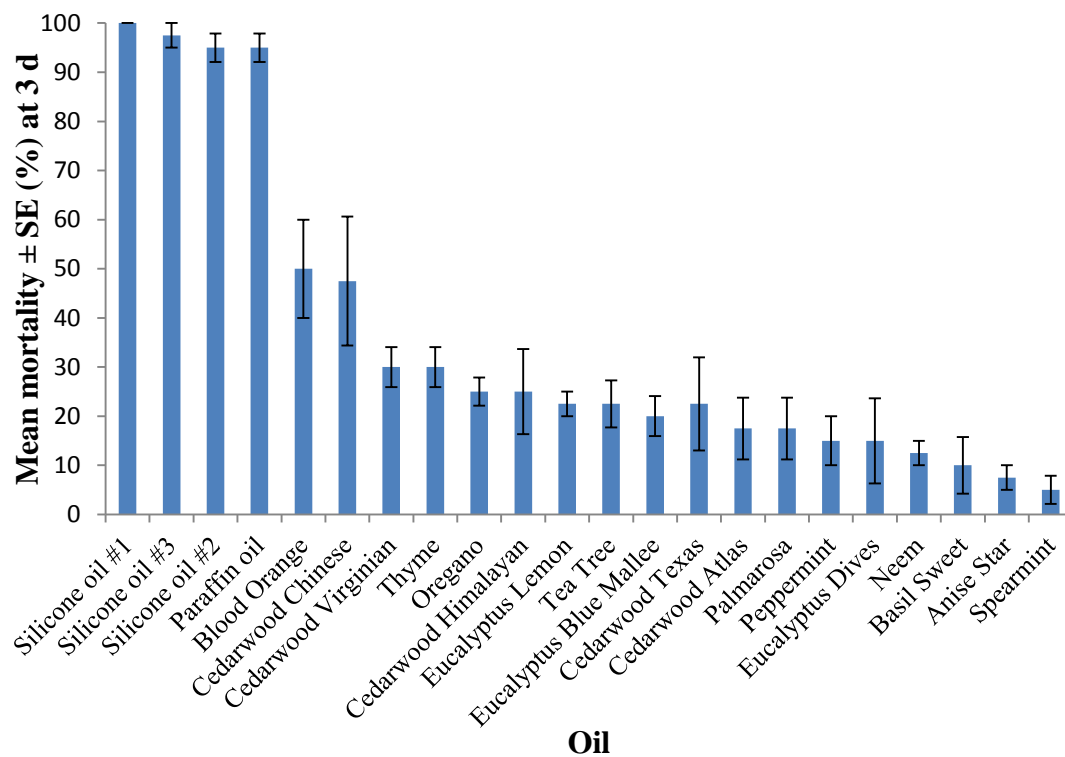


Fig. 1. Mortality of bed bugs after topically treated with different oils at the dose of 0.1 mg oil per bug, arranged in order by mean rank scores. There was no mortality in the control.

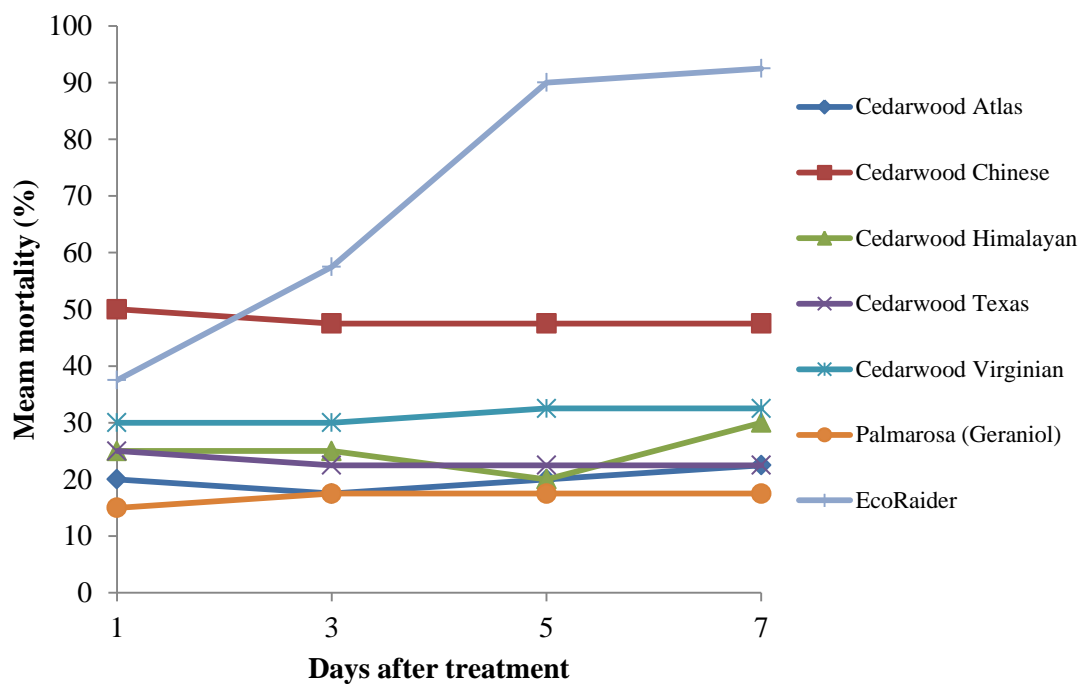


Fig. 2. Mortality of bed bugs treated with different cedarwood oils, geraniol, and EcoRaider at the dose of 0.1 mg oil or EcoRaider original solution (5 ng each active ingredient in 0.5 μ l) per bug. There was no mortality in the control.

Chapter Four

Effect of Moxidectin on Bed Bug Feeding, Development, Fecundity, and Survivorship

Abstract

The common bed bug, *Cimex lectularius* L. (Hemiptera: Cimicidae), is a blood-sucking ectoparasite which experienced world-wide resurgence during recent decades. The control of bed bugs is often challenging, due to their cryptic nature and resistance to conventional insecticides in field populations. In this study we evaluated the effect of moxidectin, an anthelmintic drug, on bed bug survival, reproduction, and development. The LC_{50} of moxidectin against bed bug male adults, female adults, and large nymphs were 52.7 (95% CI: 39.5-70.8), 29.3 (95% CI: 20.7-40.5), and 29.1 ng/ml (95% CI: 23.3-35.3), respectively. High concentrations of moxidectin reduced the fecundity of bed bug females, but showed no significant effect on egg hatching. One time feeding on rabbit blood containing 20 and 40 ng/ml moxidectin showed no negative effects on bed bug feeding and blood meal ingestion, but significantly reduced digestion rates and nymph molting rates. Although moxidectin in medium concentrations (20-40 ng/ml) only caused moderate mortality in bed bugs, it significantly interrupted digestion, development, and oviposition of surviving bed bugs for at least 1 wk after feeding. Therefore, moxidectin treatment is a promising supplement of the existing bed bug management IPM if its use on humans can be approved in the future.

Introduction

The common bed bug, *Cimex lectularius* L. (Hemiptera: Cimicidae), is a blood-sucking ectoparasite which experienced world-wide resurgence during recent decades

(Doggett et al. 2004, Romero et al. 2007, Wang and Wen 2011, Davies et al. 2012). Bed bug bites cause itchiness, rashes, anxiety, sleeplessness, and other psychological sequelae (Hwang et al. 2005, Goddard and de Shazo 2012); people who had little or no reaction to bed bug bites can be sensitized after repeated exposure (Reinhardt et al. 2009). Public tolerance of bed bugs is almost zero, and social stigma and economic hardship are often associated with bed bug infestations (Potter 2006). Control of bed bugs is often challenging. One reason is that they are good at hiding, therefore very thorough inspections and treatments are required for successful management (Potter 2006). Another factor is that resistance to pesticides has been widely established in bed bug field populations. As a result of regulatory restrictions on many conventional insecticides such as DDT, carbamates, and organophosphates, pyrethroids have become the mainstay chemicals used for bed bug control over the past decades (Davies et al. 2012). However, resistance to pyrethroids is widespread in bed bug populations across the United States (Romero et al. 2007, Zhu et al. 2010, Davies et al. 2012). Despite the development of many bed bug control methods and materials (Wang et al. 2009, Wang et al. 2011, Cooper et al. 2016), new and effective treatment materials and methods are needed to improve the efficacy of current bed bug management programs.

The potential of anthelmintic drugs in killing bed bugs was recently studied as a new concept in bed bug control (Sheele et al. 2013). Sheele and Ridge (2016) fed bed bugs with blood containing ivermectin and moxidectin, and found that blood treated with higher concentrations of > 25 ng/mL ivermectin or moxidectin caused significantly higher mortality (50–100 %) in bed bugs than the controls (0–6 %) by day 13 (Sheele and Ridge 2016). Besides direct mortality, bed bugs treated with ivermectin and moxidectin

also showed signs of sub-lethal effects including reduced fecundity, feeding difficulty, and incomplete ecdysis.

Moxidectin is derived from nemadectin, an active fermentation milbemycin product isolated from *Streptomyces cyanogriseus* in 1983 (Prichard et al. 2012). It has been widely used in cattle, sheep, and companion animals for parasite treatments (Wagner and Wendlberger 2000, Dupuy et al. 2003). It is effective against a wide range of parasites including cyathostomes and other equine parasites (Xiao et al. 1994), *Muellerius capillaris* (hair lungworm) (Vadlejch et al. 2016), ascarids, strongyles, tapeworms (Lyons et al. 2017), sarcoptic, demodectic, and psoroptic mites (Wagner and Wendlberger 2000), as well as human lymphatic filarial parasite (Verma et al. 2014). Based on previous studies on nematodes, the primary mechanism of moxidectin is binding to invertebrate-specific glutamate-gated chloride channels, leading to paralysis and death of the parasite due to hyperpolarization of nerves and muscle fibers (Cully et al. 1994, Forrester et al. 2002). Although not yet approved for human use, moxidectin is safe and well tolerated in humans in a single oral dose between 3-36 mg (Cotreau et al. 2003). It is currently being tested for treating onchocerciasis (river blindness) in Africa, with positive results in both safety and efficacy (Awadzi et al. 2014). Moxidectin is also a promising treatment for human scabies (Mounsey et al. 2016).

The dynamic of oral moxidectin in human plasma has been studied in details by Cotreau et al. 2003. For doses between 3-36 mg, the time of maximum moxidectin plasma concentration varied from 1.8-4 h, with a very long half-life in plasma (20-35 d). After a single oral dose of 36 mg, the concentration of moxidectin in plasma was >10 ng/mL for the first 8 days, and then dropped to 1-10 ng/mL during the following 8-80

days. Unlike internal parasites, bed bugs will not be exposed to anthelmintic drugs in blood plasma right after host's oral ingestion; it may take hours, or even days, before bed bugs start host seeking and feeding after the drugs are taken. Therefore, the long half-life and stability in plasma makes moxidectin a more promising drug against bed bugs than those with short half-lives such as ivermectin (18-22 h) (Sheele and Ridge 2016).

Although the effect of moxidectin on bed bugs was reported in a previous study (Sheele and Ridge 2016), information on the toxicity and sub-lethal effects of moxidectin is still very limited due to small sample size. In this study, we fed bed bugs with rabbit blood containing different concentrations of moxidectin, and then evaluated its effect on bed bug feeding, development, fecundity, and survivorship. We determined the LC_{50} of moxidectin against different sexes and stages of bed bugs, measured the impacts of moxidectin on bed bug feeding, ingestion, and digestion. We also determined the effects of moxidectin on female oviposition, and egg and nymph development.

Materials and Methods

Chemicals. Cydectin[®] Injectable Moxidectin (Boehringer Ingelheim Vetmedica Inc., Duluth, GA) containing propylene glycol (50–75%), ethanol (20%), and 1% moxidectin was used in moxidectin treatment groups. Hanks balanced salt solution (HBSS) was used as buffer solution for dissolving 1% moxidectin stock solution before mixing with blood.

Insects. A field strain of bed bug (Irvington strain) was used in this study. It was collected from multiple apartments during 2012-2013 in a building in New Jersey, and was moderately resistant to pyrethroid insecticides in our preliminary laboratory assay in January 2016. Bed bugs were maintained in plastic containers (5 cm diameter and 4.7 cm

height; Consolidated plastics, Stow, OH) with folded construction paper (40 mm length and 30 mm width; Universal Stationers Supply Co., Deerfield, IL) as harborages and held at $26 \pm 1^\circ\text{C}$, $40 \pm 10\%$ relative humidity (RH), and a 12:12 h L:D photoperiod. They were fed biweekly on defibrinated rabbit blood (Hemostat Laboratories, Dixon, CA) using a Hemotek membrane-feeding system (Discovery Workshops, Accrington, UK). Rearing containers were modified into feeding jars by cutting the bottom off and attaching a fine nylon mesh through which bed bugs can feed on the blood source. Bed bugs were fed 1 wk prior to all experiments.

Experiment 1: Effect of moxidectin on bed bug morality, female fecundity, and egg development. Engorged bed bug males, females, and nymphs were selected after feeding, and were used in experiments after 1 wk. Third to 5th instar nymphs were selected except engorged 5th instars which close to molt to avoid eclosion during the experimental period. Different sexes and stages were contained separately in individual feeding jars. The numbers of males, females, and nymphs included in each trial were 15, 15, and 30, respectively. During the experiment, bed bugs were fed with blood containing the following concentrations of moxidectin: 2.5, 5, 25, 50, 100, and 150 ng/ml. Moxidectin stock solution (1%) was diluted in HBSS buffer solution before mixing with blood; the final mixture contained 99.96% blood and 0.04% HBSS and moxidectin. Control groups were fed with blood containing the same volume of HBSS only. Each treatment was replicated 3 times (N=45, 45, and 90 for males, females, and nymphs, respectively).

Bed bugs were fed for 30 min under room temperature; the numbers of engorged bed bugs were recorded, and unfed bed bugs were removed and excluded from the study.

Females were moved to new harborages for oviposition observation. Bed bugs were kept in an incubator (Percival Scientific Inc., Perry, IA) maintained at $26 \pm 1^{\circ}\text{C}$, $40 \pm 10\%$ relative humidity (RH), and a 12:12 h L:D photoperiod after treatment. Mortalities were recorded after 1 wk, and eggs laid by females during the 7-d period were observed for 10 d for hatching rates.

Experiment 2: Effect of moxidectin on bed bug feeding, digestion, and nymph development. Bed bugs were selected and prepared as in Experiment 1 and fed with rabbit blood containing 20 and 40 ng/ml moxidectin. The two concentrations were chosen to present moderate moxidectin levels in human blood plasma after a single 36-mg oral dose within the first week (Cotreau et al. 2003). Control groups were fed with rabbit blood containing the same volume of HBSS only. Treated rabbit blood and bed bugs were prepared following the same protocol as in Experiment 1. The numbers of males, females, and nymphs included in each trial were 15, 15, and 30, respectively. Each treatment was replicated 3 times.

Bed bugs were fed for 30 min under room temperature, the numbers of engorged bed bugs were recorded, and unfed bed bugs were excluded from the study. Bed bugs were kept in an incubator after treatment as in Experiment 1. Bed bug males and females were weighed on a balance (Mettler-Toledo Inc., Washington Crossing, PA) in groups of 15 before feeding. After feeding, only engorged bed bugs were weighed. The mortalities were recorded after 1 and 2 wk, and live bed bugs were again weighed in groups.

Nymphs treated in Experiment 2 were observed for molting at 1 and 2 wk after feeding. The numbers of exuviae and live nymphs were recorded to evaluate the molting

rate. The feeding and digestion rates of nymphs were not evaluated because the size of the nymphs varied.

The visual appearances of treated bed bugs were observed; bed bugs that killed by moxidectin and bed bugs that survived in 40 ng/ml moxidectin treatment after 1 wk were recorded by camera and compared with control.

Experiment 3: Repeated feeding on moxidectin treated blood. Surviving bed bugs in Experiment 2 were again fed with rabbit blood containing the same moxidectin concentration at 2 wk after the initial treatment, and the experimental protocols in Experiment 2 were repeated. The experiment stopped after 2nd feeding because there were not enough survived bed bugs to continue.

Data analysis. Probit analysis was used to calculate LC₅₀ values of moxidectin against bed bug males, females, and nymphs. Since the lower concentrations (2.5-50 ng/ml) and higher concentrations (100 and 150 ng/ml) were tested separately with controls, all mortalities were adjusted by Abbott's formula before Probit analysis. The average mortalities in controls were less than 5%. The numbers of eggs laid per survived female and egg hatching rates in different concentrations of moxidectin treatments were compared by Analysis of Variance (ANOVA) followed by Tukey's HSD test ($P < 0.05$).

Effect of moxidectin on bed bug feeding and digestion was evaluated by calculating feeding rate, ingestion rate, and digestion rate:

$$\text{Feeding rate} = \frac{\text{number of engorged bed bugs after feeding}}{\text{number of bed bugs before feeding}} \times 100\%$$

$$\text{Ingestion rate} = \frac{\text{average weight gain after feeding}}{\text{average body weight before feeding}} \times 100\%$$

Digestion rate

$$= \frac{\text{average weight after feeding} - \text{average weight after digesting period}}{\text{average weight gain after feeding}}$$

× 100%

Feeding rates, ingestion rates, and digestion rates of bed bugs fed on 0, 20, and 40 ng/ml moxidectin were analyzed by ANOVA followed by Tukey's HSD test. In Experiment 2, the digestion rate of females fed on 40 ng/ml moxidectin was not analyzed because the number of surviving bed bugs in some groups was too small (< 5). In that case, bed bugs fed on 0 and 20 ng/ml moxidectin were compared by Student's t-test. Feeding rates of males and nymphs in Experiment 3 were analyzed by ANOVA followed by Tukey's HSD test; other indices were not analyzed because the numbers of survived bed bugs were too small (< 5) in most treated groups.

Effect of moxidectin on bed bug development was evaluated by calculating nymph molting rate:

$$\text{Nymph molting rate} = \frac{\text{Number of exuviae}}{\text{Number of survived nymphs}} \times 100\%$$

Molting rates of bed bug nymphs fed on 0, 20, and 40 ng/ml moxidectin were analyzed by ANOVA followed by Tukey's HSD test. Mortalities of bed bug males, females, and nymphs fed on control, 20 and 40 ng/ml moxidectin were compared by two-way ANOVA followed by Tukey's HSD test.

Bed bug mortalities and molting rates in Experiment 3 were summarized but not analyzed for statistical differences because the numbers of surviving and fed again in many treatment groups were too small. All analyses were conducted using SAS software 9.3 (SAS Institute 2011).

Results

Toxicity of moxidectin against bed bugs. The LC_{50} values of moxidectin against bed bug males, females, and large nymphs were 52.7 (95% CI: 39.5-70.8), 29.3 (95% CI: 20.7-40.5), and 29.1 (95% CI: 23.3-35.3) ng/ml, respectively (Table 1). The LC_{50} value for nymphs was significantly lower than that for males based on non-overlapped 95% CIs.

Effect of moxidectin on bed bug female fecundity and egg hatching. The number of eggs laid per survived female decreased significantly with the increase of moxidectin concentration ($F = 101.22$; $df = 4, 10$; $P < 0.001$). Eggs laid per female that fed with 5 ng/ml moxidectin (7.6 ± 0.9) was significantly less than control and 2.5 ng/ml moxidectin treatment (11.0 ± 0.7 and 10.5 ± 0.4 , respectively); eggs laid per female that fed with 25 and 50 ng/ml moxidectin (0.6 ± 0.1 and 0 ± 0 , respectively) were significantly less than 5 ng/ml moxidectin treatment (Tukey's HSD test, $P < 0.05$). However, the egg hatching rate were not significantly affected by moxidectin treatments ($F = 0.29$; $df = 3, 8$; $P = 0.835$) (Table 2).

Effect of moxidectin on bed bug feeding, ingestion, and digestion. The feeding rates, ingestion rates, and digestion rates of bed bugs fed on untreated and treated blood are shown in Table 3.

The feeding rates of males on blood containing 0, 20, and 40 ng/ml moxidectin were 98 ± 2 , 84 ± 13 , $84 \pm 6\%$, respectively. There was no significant difference among control and treatment groups ($F = 0.92$, $df = 2, 6$; $P = 0.447$). The feeding rates of females on blood containing 0, 20, and 40 ng/ml moxidectin were 96 ± 5 , 93 ± 0 , $96 \pm 2\%$, respectively. There was no significant difference among control and treatment groups ($F = 0.21$, $df = 2, 6$; $P = 0.820$). The feeding rates of nymphs on blood containing 0, 20, and

40 ng/ml moxidectin were 97 ± 2 , 93 ± 2 , $89 \pm 6\%$, respectively. There was no significant difference among control and treatment groups ($F = 1.08$, $df = 2, 6$; $P = 0.397$).

The ingestion rates of males on blood containing 0, 20, and 40 ng/ml moxidectin were 44 ± 1 , 57 ± 2 , $57 \pm 1\%$, respectively. The ingestion rates in treatment groups (20 and 40 ng/ml moxidectin) were significantly higher than control ($F = 24.00$; $df = 2, 6$; $P = 0.001$. Tukey's test, $P < 0.05$). The ingestion rates of females on blood containing 0, 20, and 40 ng/ml moxidectin were 70 ± 3 , 75 ± 0 , $73 \pm 0\%$, respectively. There was no significant difference among control and treatment groups ($F = 2.79$, $df = 2, 6$; $P = 0.139$).

The digestion rates of males at 1 wk after feeding on blood containing 0, 20, and 40 ng/ml moxidectin were 64 ± 3 , 50 ± 3 , and $25 \pm 5\%$, respectively. The digestion rate in 40 ng/ml moxidectin treatment was significantly lower than control and 20 ng/ml moxidectin treatment ($F = 25.33$; $df = 2, 6$; $P = 0.001$. Tukey's test, $P < 0.05$). The digestion rates of females at 1 wk after feeding on blood containing 0 and 20 ng/ml moxidectin were 78 ± 3 and $31 \pm 2\%$, respectively. The digestion rate of 20 ng/ml moxidectin treatment was significantly lower than control ($t = 13.81$; $df = 4$; $P < 0.001$). The digestion rates of females in 40 ng/ml moxidectin treatment were not analyzed due to low survivorship.

The digestion rates of males at 2 wk after feeding on blood containing 0, 20, and 40 ng/ml moxidectin were 95 ± 4 , 95 ± 3 , and $70 \pm 4\%$, respectively. The digestion rate of 40 ng/ml moxidectin treatment was significantly lower than control and 20 ng/ml moxidectin treatment ($F = 19.21$; $df = 2, 6$; $P = 0.003$. Tukey's test, $P < 0.05$). The digestion rates of females at 2 wk were not analyzed due to low survivorship.

Effect of moxidectin on nymph development. There were 86, 58, and 39 nymphs that survived at 2 wk after feeding on blood containing 0, 20, and 40 ng/ml moxidectin, respectively. The nymph molting rates at 1 wk after feeding on blood containing 0, 20, and 40 ng/ml moxidectin were 99 ± 1 , 3 ± 2 , and $0 \pm 0\%$, respectively. The molting rates of nymphs fed on 20 and 40 ng/ml moxidectin were significantly lower than control ($F = 2067.22$; $df = 2, 6$; $P < 0.001$. Tukey's HSD test, $P < 0.05$).

The nymph molting rates at 2 wk after feeding on blood containing 0, 20, and 40 ng/ml moxidectin were 100 ± 0 , 84 ± 8 , and $48 \pm 16\%$, respectively. The molting rate of nymphs fed on 40 ng/ml moxidectin were significantly lower than control and 20 ng/ml moxidectin treatment, but there was no significant difference between control and 20 ng/ml moxidectin treatment ($F = 6.87$; $df = 2, 6$; $P = 0.028$. Tukey's HSD test, $P < 0.05$).

Differential susceptibility of males, females, and nymphs to moxidectin. At 1 wk after treatment, the mortalities of bed bug males, females, and nymphs in control groups were 5 ± 2 , 2 ± 2 , and $0 \pm 0\%$, respectively. The mortalities of bed bug males, females, and nymphs fed on 20 ng/ml moxidectin were 7 ± 5 , 50 ± 9 , and $30 \pm 4\%$, respectively. The mortalities of bed bug males, females, and nymphs fed on 40 ng/ml moxidectin were 31 ± 7 , 82 ± 5 , and $47 \pm 3\%$, respectively.

The mortalities of bed bugs in different treatments were significantly different ($F = 97.48$; $df = 2, 18$; $P < 0.001$). There were also significant differences among bed bugs in different sexes and stages ($F = 29.80$; $df = 2, 18$; $P < 0.001$). There was an interaction between treatments and bed bug sexes/stages ($F = 8.8$; $df = 4, 18$; $P < 0.001$). The mortality of females that were fed with 20 ng/ml was significantly higher than male; the

mortality of females that were fed with 40 ng/ml was significantly higher than males and nymphs (Tukey's HSD test, $P < 0.05$).

Visual changes of bed bugs after moxidectin ingestion. Bed bugs that died after ingesting moxidectin treated blood are shown in Fig. 1. Intoxicated bed bugs became motionless and paralyzed at first, and gradually died in relaxed positions. Separation of clear liquid and black matter in the abdomen was observed, possibly due to coagulation of indigestible blood in the gut.

Bed bugs that survived at 1 wk after ingesting control blood and blood containing 40 ng/ml moxidectin are shown in Fig. 2. Compared to control groups, bed bugs fed with moxidectin were still engorged with blood, indicating suppressed digesting process.

Effect of repeated moxidectin treatment. During the 2nd treatment, the feeding rates of bed bug males fed on control, 20, and 40 ng/ml moxidectin were 95 ± 3 , 75 ± 18 , and $43 \pm 17\%$, respectively. There was no significant difference among control and treatments ($F = 3.59$; $df = 2, 6$; $P = 0.095$). The feeding rates of bed bug nymphs fed on control, 20, and 40 ng/ml moxidectin were 37 ± 8 , 55 ± 5 , and $17 \pm 17\%$, respectively. There was no significant difference among control and treatments ($F = 2.99$; $df = 2, 6$; $P = 0.126$). The feeding rates of females were not analyzed due to low survivorship.

At 1 wk after 2nd treatment, the mortalities of males fed on control, 20, and 40 ng/ml moxidectin were 0, 16, 10%, respectively. The mortalities of females fed on control, 20, and 40 ng/ml moxidectin were 11, 0, 0%, respectively. The mortalities of nymphs fed on control, 20, and 40 ng/ml moxidectin were 3, 28, 0%, respectively.

At 2 wk after 2nd treatment, the mortalities of males fed on control, 20, and 40 ng/ml moxidectin were 0, 32, 10%, respectively. The mortalities of females fed on

control, 20, and 40 ng/ml moxidectin were 14, 11, 100%, respectively. The mortalities of nymphs fed on control, 20, and 40 ng/ml moxidectin were 3, 28, 0%, respectively. The mortalities were not statistically analyzed because the survivorships in some groups were low (< 3 bed bugs), and some treatment had less than 3 replications left (Table 5).

Discussion

Based on the previous work by Sheele and Ridge (2016) on the lethal and sub-lethal effects of moxidectin against bed bugs, I further analyzed the toxicity of moxidectin. I determined the LC_{50} values of moxidectin against different bed bug sexes and stages, and quantified sub-lethal effects on various physiological indices including female fecundity, hatching rate, molting rate, feeding, ingestion rate, and digestion rate.

Moxidectin showed insecticidal activity against bed bugs when ingested in a blood meal. Among different bed bug sexes and stages, males were less susceptible to moxidectin treatment. Beside immediate insecticidal activity, moxidectin also reduced the fecundity of bed bug females. The number of eggs laid per survived female decreased significantly when the concentration of moxidectin increased. No oviposition occurred during 1 wk after feeding when the concentration of moxidectin reached 50 ng/ml. On the other hand, the hatching rates were not significantly affected by moxidectin.

No feeding deterrence of moxidectin was found during the feeding experiment at effective concentrations. In fact, the males in treated groups ingested more blood meal than the control group, while there was no significant difference among females that received different treatments. The reason for lower ingestion rate among males in the control was not clear, further experiment with larger sample size is needed to explain this phenomenon.

Although moxidectin did not show any negative effects in bed bug feeding and blood meal ingestion, bed bugs that ingested moxidectin suffered from decreased digestion. Previous studies show that the mode of action of moxidectin in killing nematodes is binding to invertebrate-specific glutamate-gated chloride channels, leading to paralysis and death due to hyperpolarization of nerves and muscle fibers (Cully et al. 1994, Forrester et al. 2002). Our observation in bed bugs was congruent with that conclusion: treated bed bugs became paralyzed and died slowly with blood meals undigested; survived bugs could not digest, molt, or lay eggs probably due to suppressed muscle and neuro activities. At 1 wk after feeding on treated blood, survived bed bug males, females, and nymphs were still engorged, while bed bugs fed on untreated blood had already digested the blood meals with abdomens flattened, indicating a result of interrupted digestion. Further studies using molecular biology methods are needed to determine if the mode of action of moxidectin in bed bugs is the same as in nematodes.

Moxidectin also slowed down the development of nymphs. At 1 wk after treatment, the molting rates of bed bug nymphs in 20 and 40 ng/ml moxidectin treatments were significantly lower than the control. At 2 wk after treatment, the molting rate of bed bug nymphs in 40 ng/ml moxidectin treatment was still significantly lower than control, while the molting rate in 20 ng/ml moxidectin treatment increased to the same level as control, indicating that bed bug nymphs fed on 20 ng/ml moxidectin could recover after 2 wk.

At 2nd feeding, the feeding rates of males and nymphs in control and treatments were not significantly different; the relatively low feeding rates in nymphs were likely caused by environment factors since the control feeding rate was low as well. The

mortality of re-treated bed bugs were low (Table 4), and most treated nymphs were able to molt after 2 wk (Table 5). These results indicate that after repeated moxidectin treatment, some bed bugs will still be able to survive, recover, or may even show resistance against moxidectin. Further feeding experiment in large scale is needed to determine the long-term sub-lethal effects and resistance of bed bugs against moxidectin.

Since high concentrations of moxidectin in blood are needed to achieve higher mortality (> 90%) in bed bugs, it is unlikely that bed bugs can be eliminated by taking moxidectin drug alone. Nevertheless, moxidectin in moderate concentrations (20-40 ng/ml) not only caused direct mortality to bed bugs, but also significantly suppressed the digestion, development, and oviposition of survived bed bugs for at least 1 wk after feeding. Unlike most other bed bug control methods, taking moxidectin requires little, if any, efforts for residents. Moxidectin treatment is a promising method in bed bug IPM programs. Because bed bugs will keep engorged for longer period after moxidectin ingestion, they may be more susceptible to other treatments such as insecticide application or steam application. Future studies on the interactions between moxidectin treatment and other treatment methods would help us to better understand the potential of using moxidectin in bed bug IPM.

In conclusion, moxidectin not only causes direct mortality in bed bugs, but also reduces the fitness of bed bugs by causing various sub-lethal effects, including reduced digestion, development, and fecundity. Bed bugs survived after feeding on blood containing moderate concentrations (20-40 ng/ml) of moxidectin kept engorged for at least 1 wk. Females laid significantly less eggs, and nymphs could not molt. A small portion of bed bugs could survive after repeated treatment. Therefore, moxidectin may

not be an effective bed bug control method alone, but shows potential as a supplementary method in bed bug IPM.

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Table 1. Probit analysis results of toxicity of moxidectin against bed bugs.

Sex/stage	N	Slope \pm SE	LC ₅₀ (95% CI) (ng/ml)	χ^2	P
Male adult	230	2.70 \pm 0.38	52.7 (39.5-70.8)	50.4	< 0.001
Female adult	214	2.46 \pm 0.32	29.3 (20.7-40.5)	58.6	< 0.001
Nymph	332	3.93 \pm 0.42	29.1 (23.3-35.3)	88.4	< 0.001

Table 2. Effect of moxidectin on bed bug female fecundity and egg hatching.

Moxidectin treatment (ng/ml)	Control	2.5	5	25	50	ANOVA statistics
No. of survived females	30	35	31	13	14	-
Eggs laid per survived female	11.0 \pm 0.7 ^{a†}	10.5 \pm 0.4 ^a	7.6 \pm 0.9 ^b	0.6 \pm 0.1 ^c	0 \pm 0 ^c	F = 101.22; df = 4, 10; P < 0.001
Egg hatching rate%	95 \pm 2	89 \pm 2	90 \pm 4	83 \pm 17	-	F = 0.29; df = 3, 8; P = 0.835

†Numbers followed by different letters are significantly different (Tukey's HSD test, P < 0.05).

Table 3. Effect of moxidectin on bed bug feeding, ingestion, and digestion.

Moxidectin treatment (ng/ml)		Control	20	40	ANOVA statistics
Feeding%	Male	98 ± 2	84 ± 13	84 ± 6	F = 0.92, df = 2, 6; P = 0.447
	Female	96 ± 5	93 ± 0	96 ± 2	F = 0.21, df = 2, 6; P = 0.820
	Nymph	97 ± 2	93 ± 2	89 ± 6	F = 1.08, df = 2, 6; P = 0.397
Ingestion%	Male	44 ± 1 ^{b†}	57 ± 2 ^a	57 ± 1 ^a	F = 24.00; df = 2, 6; P = 0.001
	Female	70 ± 3	75 ± 0	73 ± 0	F = 2.79, df = 2, 6; P = 0.139
Digestion% after 1 wk	Male	64 ± 3 ^a	50 ± 3 ^a	25 ± 5 ^b	F = 25.33; df = 2, 6; P = 0.001
	Female	78 ± 3 ^a	31 ± 2 ^b	-	t = 13.81; df = 4; P < 0.001
Digestion% after 2 wk	Male	95 ± 4 ^a	95 ± 3 ^a	70 ± 4 ^b	F = 19.21; df = 2, 6; P = 0.003
	Female	-	-	-	-

† Numbers within the same row followed by different lower case letters are significantly different (Tukey's HSD test, P < 0.05).

Table 4. Mortalities of bed bugs after the 2nd moxidectin treatment.

Sex/stage	Moxidectin treatments	n†	Mortality at 1 wk (%)	Mortality at 2 wk (%)
Male adult	Control	39	0	0
	20 ng/ml	25	16	32
	40 ng/ml	10	10	10
Female adult	Control	28	11	14
	20 ng/ml	9	0	11
	40 ng/ml	1	0	100
Nymph	Control	32	3	3
	20 ng/ml	32	28	28
	40 ng/ml	6	0	0

† The number included those fed and survived the 1st moxidectin treatment, and fed during the 2nd moxidectin treatment.

Table 5. Percentages of bed bug nymphs molted after the 2nd moxidectin treatment.

Moxidectin treatments	n†	Percent molted after 1 wk	Percent molted after 2 wk
Control	32	87	87
20 ng/ml	32	0	83
40 ng/ml	6	50	83

† The number included those fed and survived the 1st moxidectin treatment, and fed during the 2nd moxidectin treatment.



Fig. 1. Bed bugs died after ingesting moxidectin treated rabbit blood.

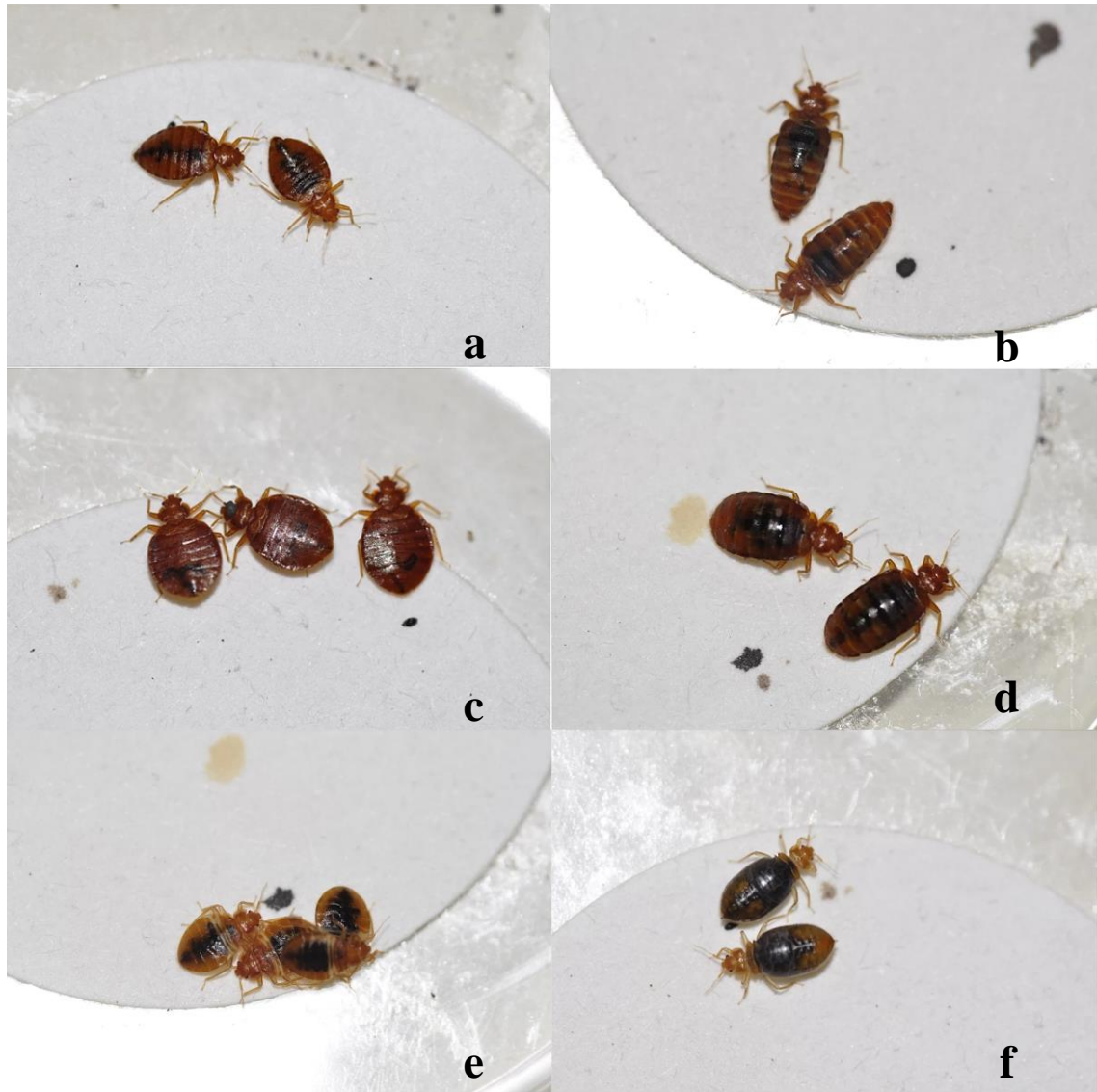


Fig. 2. Bed bugs survived at 1 wk after ingesting untreated rabbit blood and rabbit blood containing 40 ng/ml moxidectin. a) control males; b) treated males; c) control females; d) treated females; e) control nymphs; f) treated nymphs.

CONCLUSION

Community-wide IPM implementation is not only highly effective for reducing cockroach population and eliminating infestations, but also for reducing indoor insecticide residue levels. After 7 mo IPM implementation in a low-income community in New Brunswick, NJ, 85% of the cockroach infestations found during the initial inspection were eliminated. Most residents in our survey stopped self-application of insecticides after our treatment and education. Both the number and concentration of detected target pyrethroid residues were reduced significantly after 7 mo IPM implementation.

The cockroach infestations within a multi-unit dwelling at Paterson, NJ were not independent to each other before IPM implementation. The correlation among infested apartments was weakened at 6 mo after initial treatment, and infestations in the building were independent to each other at 12 mo after initial treatment. Newly established infestations at 6 and 12 mo were independent to infestations that existed 6 mo ago. The results indicate that building-wide cockroach IPM implementation not only reduce cockroach populations and eliminate infestations, but also weaken the correlation among infestations. They also imply that once a German cockroach infestation becomes established in an apartment, cockroaches tend to spread to the neighboring units. Therefore, early detection and prompt treatment is important for designing cockroach management programs to minimize cost and the negative impact of cockroach infestations. Compared to cockroach monitoring trap placed for 2 d, 14 d trap placement showed better performance in detecting cockroach infestations; the failure rate of 2 d trap placement in detecting existing infestation was as high as 52% at 7-9 wk after initial

treatment. Therefore, it would be much more accurate if traps are placed for at least a 14 d period in cockroach IPM programs than using a 2 d trapping period.

Various essential oils, silicone oils, and paraffin oil showed different levels of toxicity against bed bugs in topical assays, while silicone oils and paraffin oil were generally more effective than essential oils. One silicone oil water mixture tested in the study caused high mortality to bed bugs via direct spray at low concentration (1%). Considering the fact that most “green pesticides” against bed bugs on the market are essential oil products, silicone oils and paraffin oil are promising new candidates for low-risk consumer products against bed bugs.

Moxidectin, an anthelmintic drug widely used on pets and livestock, showed toxicity against bed bugs when ingested with blood meals. Besides direct mortality, moxidectin caused various sub-lethal effects on survived bed bugs, including delayed molting, reduced fecundity, and reduced digestion rate. Survived bed bugs ingested 20 or 40 ng/ml moxidectin kept engorged for 1 wk, which reduced their fitness by interrupting digestion, development, and oviposition. It is plausible to suggest that host seeking and dispersal of treated bed bugs would be interrupted as well under field conditions. Engorged bed bugs after treatment may be also more susceptible to other treatments. Therefore, moxidectin treatment is a promising supplement of the existing bed bug management IPM if its use on humans can be approved in the future.

Overall, my studies determined and evaluated multiple new approaches in cockroach and bed bug IPM. The results of these studies will help in: 1) further understanding of the benefits of IPM implementation in urban pest control, 2) adopting more accurate and reliable cockroach monitoring methods, 3) developing new low-risk

pest control materials, and 4) developing new bed bug management approaches by using anthelmintic drug.