Chapter 8
Development of Semiochemicals and Diatomaceous Earth Formulations for Bed Bug Pest Management

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Abstract Bed bugs are obligate blood feeders on humans. In recent years, bed bug, *Cimex lectularius* L., (Hemiptera: Cimicidae) infestations have increased dramatically in many parts of the world including Canada and the USA, leading to a renewed interest in the chemical ecology of these pests to design better control options. According to Health Canada, bed bugs can now be found everywhere from homeless shelters to five-star hotels and from single-family dwellings to public transportation. Given that bed bugs are among the most difficult pests to eradicate, along with their demonstrated resistance to conventional insecticides and ease of transport, the key objective of our research is to facilitate the development of products for management of bed bugs, based on semiochemicals – nontoxic behavior-modifying substances or natural products such as diatomaceous earth. A more thorough understanding of how such chemicals influence bed bugs will inform the most effective uses of the formulated products as part of a bed bug pest management system. Although the consumer market is currently flooded with products of dubious composition and efficacy, these products are rarely adopted by pest management practitioners due to the lack of scientific data supporting claims of control. Our research involves helping our industry partners advance to the forefront in the development of safe and effective products for management of these public health pests. We have identified lead compounds as repellents as well as attractants and have developed specific diatomaceous earth (DE) dust formulations as part of a bed bug management strategy.

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8.1 Introduction

Bed bugs (*Cimex lectularius* L.; Hemiptera: Cimicidae) have reemerged as important public health pests in the past 15 years, with increasing intensity of urban infestations in Canada, the USA, western Europe, and Australia (Doggett et al. 2004; Potter et al. 2006; Harlan 2006). Low-income communities are more likely to suffer chronic and increased bed bug infestations due to limited financial resources available to provide effective community-wide management of infestations (Wang et al. 2011). This pest has a negative impact on the hospitality industry due to adverse publicity and litigation by persons who are bitten while staying in hotel rooms (Doggett et al. 2004; Potter et al. 2006). Since bed bugs can arrive on clothing or in suitcases of guests from infested homes or other hotels harboring the pests, hotels may become heavily infested with bed bugs (MedicineNet 2013). In addition to hotels, bed bugs have been found in movie theaters, office buildings, apartments, single-family dwellings, college dormitories, health-care facilities, laundries, shelters, transportation vehicles, and other locations where people congregate (MedicineNet 2013; Hwang et al. 2005). Bed bugs prompted the closure of the New Westminster, Canada, Public Library for 48 h in 2011 following the discovery of bed bugs in books (newwestcity 2011). At the Vancouver (Canada) Public Library, bed bugs were discovered across 12 of the Library's 22 locations in 2012 (Woo 2012). More than a third of pest management companies in the USA have treated bed bug infestations in hospitals in 2012, 6 % more than the year before and more than twice as many as in 2010, according to a survey released by the National Pest Management Association (Wjeczner 2013). The percentage of exterminators dealing with bed bugs in nursing homes also almost has doubled since 2010, to 46 % (Wjeczner 2013).

The exact cause of this resurgence is unclear, but may be a consequence of (i) the development of resistance in bed bugs to commonly used domestic insecticides; (ii) increased human movement – both travel and migration – (iii) more frequent exchange of secondhand furnishings among homes; (iv) decreased public awareness; and (v) global warming (Harlan 2006; Romero et al. 2007; Reinhardt and Silva-Jothy 2000). "Bed bugs are making a comeback. People now travel more than ever before, and bed bugs are hitching rides on clothing and luggage. Anyone can get an infestation of bed bugs and this does not mean a lack of cleanliness" (Health Canada 2013). The US Centers for Disease Control and Prevention (CDC) and the US Environmental Protection Agency (EPA) consider bed bugs a pest of "significant public health importance" and an emerging public health problem across the USA (CDC and EPA 2010).

Bed bugs are obligate blood feeders that require blood meals for growth and development throughout their life cycle (Fig. 8.1). Bed bug nymphs typically take 4–5 weeks to complete development and reach sexual maturity (Omori 1941). Blood feeding can result in allergic reactions in human beings due to the presence of vasodilatory substances (nitric oxide) in bed bug saliva Weeks et al. 2010;

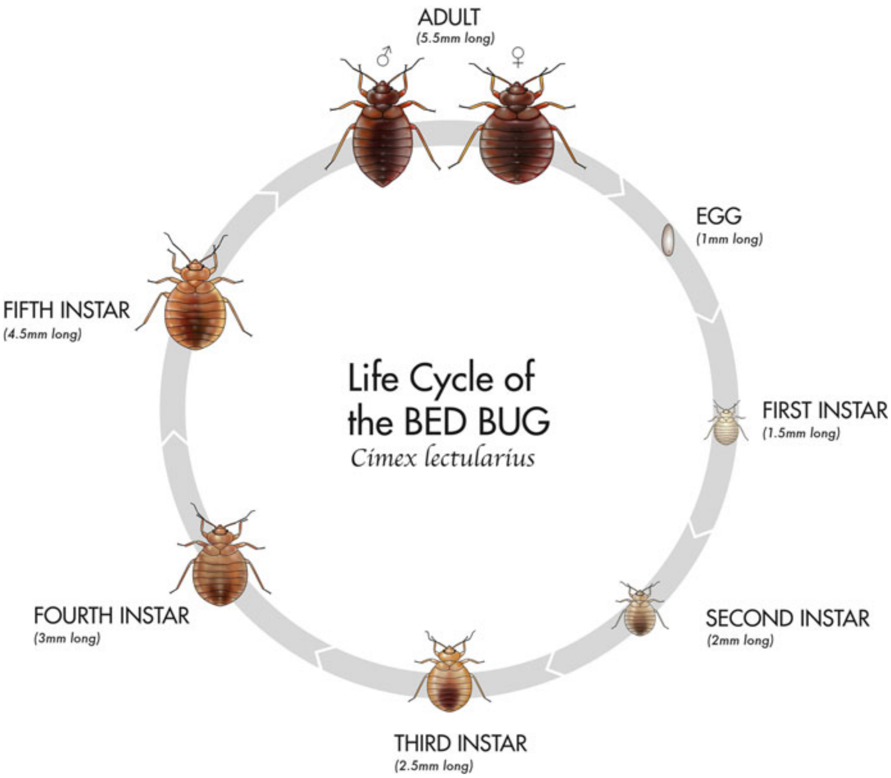

Fig. 8.1 Life cycle of *Cimex lectularius*

Doggett et al. 2012). Although there is no direct evidence that they can transmit disease between human hosts, they cause a range of emotional problems, discomfort, anxiety, and sleeplessness (Reinhardt and Silva-Jothy 2000; Doggett et al. 2012). Bed bug infestations often require expensive ongoing inspections and treatments, disposal and replacement of infested beds and other furnishings, and quarantine of infested areas (Romero et al. 2007). Treatment of bed bug infestations cost consumers in North America over $2 billion in 2010 alone. There is an urgent need to develop pest management tools that are not only effective in suppressing bed bug populations, but that do not themselves have undue negative impacts on human health (Haynes et al. 2010; Berg 2010). The latter is imperative given that bed bugs most often occur in and around bedding (up to 85 % of the population in infested rooms) (Wang et al. 2007) where humans spend up to a third of their lives. We discuss the major control strategies commonly used for the management of bed bugs with special emphasis on the use of natural products including diatomaceous earth and behavior-modifying substances or semiochemicals.

8.2 Bed Bug Control Strategies

8.2.1 Synthetic Insecticides

According to the EPA (2015), registered active ingredients for bed bug control include 29 chemicals: 16 pyrethrins and pyrethroids, 4 neonicotinoids, 3 inorganic compounds, chlorfenapyr, dichlorvos (DDVP), propoxur, S-hydroprene, alcohol, and neem oil. The majority are pyrethroids, which have limited field efficacy due to widespread resistance in urban bed bug populations (Romero et al. 2007; Zhu et al. 2010; Wang et al. 2014). Pyrethroid resistance (Table 8.1) in bed bugs has been reported in North America, Australia, Asia, and Europe and is widespread throughout the USA and presumably elsewhere (Romero et al. 2007; Zhu et al. 2010; Wang et al. 2014; Adelman et al. 2011; Kilpinen et al. 2011; Tawatsin et al. 2011; Dang et al. 2015; Doggett et al. 2011a; Seong et al. 2010). Several attempts have been made to characterize the mechanisms of resistance in these resurgent bed bug populations (Adelman et al. 2011). While a target site mutation, or *kdr* resistance, was identified as the primary mechanism of resistance in most resistant populations (Romero et al. 2007; Zhu et al. 2010; Yoon et al. 2008), other mechanisms of resistance such as enhanced detoxification enzyme activity also have been reported in a few cases (Romero et al. 2009a) or a combination of both (Adelman et al. 2011). Zhu et al. (2013) reported a unique resistant strategy in which resistant genes on the cuticle served to slow down the toxins from reaching target sites.

The development of pyrethroid resistance in bed bugs and the withdrawal of several effective insecticides, registered for bed bugs from the UK and US markets, have further reduced the options for control (Hwang et al. 2005). Chlorfenapyr, a prospective alternative to pyrethroids, is registered for bed bug control and is increasingly being used commercially (Moore and Miller 2006; Potter et al. 2008; Wang et al. 2009a; Romero 2011). Chlorfenapyr may not always control bed bugs in a timely manner (Moore and Miller 2006; Romero et al. 2010). Chlorfenapyr, used as a dry residue, produced >50 % mortality after 3 days of continuous exposure to bed bugs (Romero et al. 2010). Bed bugs exposed to chlorfenapyr EC-treated headboards took a longer exposure period to achieve 50 % mortality compared with synthetic pyrethroids (Moore and Miller 2006). The slower action of chlorfenapyr can be explained on the basis of its different mode of action (electron transport chain inhibitor) compared with synthetic pyrethroids (neurotoxin).

Pyrethroid resistance in bed bugs has prompted a shift to commercial insecticide products based on mixtures of a pyrethroid and a neonicotinoid by urban pest management professionals in the USA (Romero et al. 2010; Potter et al. 2012). These two classes of insecticides exhibit different modes of action (Gordon et al. 2014). Currently, the combination (pyrethroid/neonicotinoid) products are some of the most effective choices for control in the field (Romero et al. 2010; Potter et al. 2012). At least, four such combination products are being marketed for bed bugs including Temprid® (Bayer Environmental Science), Transport® (FMC Professional Solutions), Tandem® (Syngenta Professional Pest Management), and Bedlam Plus®

Table 8.1 Response of bed bugs to synthetic insecticides

Insecticide	Effect	Additional note	References
Deltamethrin	Widespread distribution of knockdown resistance mutation	L925I or V419L mutations responsible for knockdown resistance to deltamethrin	Zhu et al. (2010) and Yoon et al. (2008)
Deltamethrin	Based on the LD_{50} values, resistant ratios were ~5200-fold to deltamethrin	Bed bugs exhibit both kdr-type (L925I) and increased metabolic resistance to pyrethroid insecticides	Adelman et al. (2011)
Dust band (1 % cyfluthrin)	Mortality	Both dust band and IPM resulted in higher bed bug reduction than the control	Wang et al. (2013b)
β-Cyfluthrin	Unique resistance strategy	Resistant genes on cuticle slow down the toxins from reaching target sites	Zhu et al. (2013)
Deltamethrin and lambda-cyhalothrin	Mortality	Resistance in field populations in Kentucky and Ohio	Romero et al. (2007) and Zhu et al. (2010)
Pyrethroid/neonicotinoid	Mortality	Combination products – more lethal to bed bugs than active ingredient alone	Potter et al. (2012, 2013a)
Deltamethrin	Mortality	Field strain less susceptible than lab strain	Seong et al. (2010) and Moore and Miller (2006)
Deltamethrin	Mortality	Efficacy varies with feeding status	Potter et al. (2013a)
Deltamethrin + piperonyl butoxide	Synergistic ratio varied in different populations	P450 and other resistance mechanisms (enhanced metabolic activity) may be involved	Yoon et al. (2008)

(MGK), with the expectation that there will be more in the future. Laboratory and field reports indicate the products are more efficacious than pyrethroids alone and are now the most utilized category for bed bug treatment (Romero et al. 2010; Potter et al. 2013a).

To minimize the risk of insecticide exposure and the amount of insecticide used, Wang et al. (2013a, b) designed and evaluated a dust-treated band technique. Both laboratory and field data suggested that 1 % cyfluthrin dust-treated bands were highly effective in killing bed bugs. There was no significant difference in the final counts of bed bugs between dust-treated band and integrated pest management (IPM) treatments. A recent study (Devries et al. 2015) demonstrated the role of feeding status of bed bugs in the toxicity of deltamethrin; 21-day-starved bugs had a significantly lower LD_{50} [0.221 ng·bug^{-1}] compared with 2- and 9-day-starved bugs.

8.2.2 Behavior-Modifying Substances

Semiochemicals (behavior- and physiology-modifying chemicals) could be exploited for management of bed bugs (Logan and Birkett 2007) especially in multi-dwelling buildings including hotels and school dormitories (Weeks et al. 2010). Behavior-modifying substances can be based on natural pheromones eliciting a repellent or an attractant response by the bed bug. Alarm pheromones of the bed bug could be used as a repellent to deter bed bugs from human hosts, and aggregation pheromones could be used in traps to monitor or control bed bug populations in an infested area. Although some semiochemicals have been identified previously, our knowledge of how they mediate bed bug behavior and consequently how they could be utilized for bed bug management remains incomplete.

8.2.2.1 Use of Repellents to Deter Bed Bugs from Human Hosts

A potential strategy is the use of repellents to drive bed bugs away from places where human beings are sleeping in an effort to reduce bed bug bites. Insect repellents can be of three different types including semiochemicals (alarm pheromones), botanicals (based on plant essential oils), or synthetics (e.g., DEET).

Semiochemicals (e.g., pheromones) are chemical substances emitted by an organism that produce behavioral and physiological changes in receivers. Both adult and nymphal stages of bed bugs emit secretions that are repulsive to conspecifics. Of the ten compounds constituting *C. lectularius* nest odors, Levinson and Bar Ilan (1971) identified (*E*)-2-hexenal and (*E*)-2-octenal, secreted from dorsal abdominal glands in nymphs and metathoracic glands in adults, acting primarily as an alarm pheromone and responsible for eliciting dispersal behavior in conspecifics (Levinson et al. 1974).

Alarm pheromones are released by all stages of bed bugs under stress and have a dual function (Ryne 2009). In addition to causing conspecifics to disperse, it can also act as a mating deterrent. Homosexual mating is a common behavior in bed bugs. In order to avoid homosexual mating and abdominal injuries, newly fed nymphs secrete (*E*)-2-hexenal:(*E*)-2-octenal in a nymph-specific ratio [2:5], 4-oxo-(*E*)-2-hexenal and 4-oxo-(*E*)-2-octenal (Table 8.2), although 4-oxo-(*E*)-2-hexenal may also exert repellent effect on its own against males. Newly fed males also release alarm pheromones to signal their sex and avoid/reduce the risk of homosexual mating (Ryne 2009; Harraca et al. 2010; Liedtke et al. 2011; Feldlaufer et al. 2010). A comprehensive review of bed bug chemical ecology has been provided by Weeks et al. (2013) and Benoit (2011), among others.

Table 8.2 Response of bed bugs to semiochemicals

Semiochemicals	Effect	Additional note	References
4-oxo-(*E*)-2-hexenal and 4-oxo-(*E*)-2-octenal	Mating deterrent against *C. lectularius* and *C. hemipterus* adults	Absent in headspace collections of adults	Liedtke et al. (2011)
E)-2-hexenal, (*E*)-2-octenal, 4-oxo-(*E*)-2-hexenal, and 4-oxo-(*E*)-2-octenal	Mating deterrent	Nymphs of both species but were stage specific	Harraca et al. (2010)
1-octen-3-ol, lactic acid, heat, and CO_2 (dry ice)	Attractant (pitfall trap)	Caught 80 % of bed bugs in small arenas and 57 % of bed bugs overnight in large arenas. Also effective in infested apartments	Siljander et al. (2011)
Proprionic, butyric, valeric, octenol, and L-lactic acid + heat (37.2–42.2 °C) and CO_2	Attractant	Captured more bed bugs than other traps	Pfiester et al. (2008)
CO_2, heat, and natural lure (1-octen-3-ol, spearmint oil, and coriander oil)	Attractant (pitfall trap and experimental arena)	Chemical lure and CO_2 are essential for designing effective bed bug monitors – heat less important	Wang et al. (2011)

Commercially available insect repellents can be divided into two categories – synthetic chemicals and plant-derived essential oils. *N*, *N*-diethyl-3-methylbenzamide (DEET) has remained the most widely used insect repellent since 1957 and has demonstrated activity against a number of arthropods including mosquitoes, biting flies, chiggers, fleas, and ticks (Syed and Leal 2008; Pickett et al. 2008). However, DEET has a strong smell and dissolves certain plastic materials. Since many consumers are reluctant to apply DEET to their skin, there remains the need to develop new and safe repellent products (Fradin and Day 2002).

Most plant-based insect repellents currently available in the market are based on essential oils including citronella, peppermint, eucalyptus, lemongrass, geranium, and soybean among others. Most of the essential oil-based insect repellents available in the market provide a short protection time compared with DEET (Fradin and Day 2002). The repellent containing oil of eucalyptus marketed as Repel Lemon Eucalyptus Insect Repellent (WPC Brands) and Fite Bite Plant-Based Insect Repellent (Travel Medicine) provided a mean protection time of 120.1 ± 44.8 min against mosquitoes; the insect repellent, Herbal Armor® (All Terrain), consisting of 25 % essential oils (citronella, geranium, cedar, peppermint, lemongrass, and soybean) as active ingredients provided a protection time of 20 min or less (Fradin and Day 2002). The synthetic IR3535-based repellent and a formulation containing 23.8 % DEET provided an average protection time of 22.9 and 301.5 min, respectively, against mosquito bites in arm-in-cage studies (Fradin and Day 2002). Three repellent products based on plant essential oils including EcoSMART® insect repellent have been commercially registered against bed bugs in the USA (EcoSMART 2014) but not in Canada.

Wang et al. (2013a, b) evaluated the repellency of three commercially available insect repellents including DEET (97 % purity, Spectrum Laboratory Products Inc., Gardena, Ca), Cutter® Advanced Insect Repellent (7 % picaridin, United Industries Corporation, St. Louis, MO), and Rest Easy™ Bed Bug and Insect Control (0.5 % permethrin, Eaton, Twinsburg, OH) along with five nonregistered materials (two recently reported natural repellents, isolongifolenone and isolongifolanone [derived from *Humiria balsamifera*, a plant commonly found in South America] and three novel potential insect repellents developed by Bedoukian Research Inc., Danbury, CT, including 3-methyl-5-hexyl-2-cyclohexenone, propyl dihydrojasmonate, and γ-methyl tridecalactone) against *C. lectularius*. Cutter® Advanced Insect Repellent and Rest Easy™ Bed Bug and Insect Control were not active repellents against bed bugs; DEET provided a high level of repellency against bed bugs. A 10 % DEET, in the presence of carbon dioxide as a host cue, provided ≥94 % repellence for a period of 9 h. Although both isolongifolenone and isolongifolanone exhibited strong repellent effects against bed bugs, they were significantly less active than DEET. The three novel compounds (i.e., 3-methyl-5-hexyl-2-cyclohexenone, propyl dihydrojasmonate, and γ-methyl tridecalactone) exhibited similar levels of repellency and residual action as DEET in repelling bed bugs (Wang et al. 2013a).

8.2.2.2 Screening of Putative Bed Bug Repellents in the Laboratory

We have screened several naturally occurring semiochemicals or their structural and functional analogs (Gilbert 2014) for repellent (Table 8.3) and attractant effects (Table 8.4). Approximately 20 of 120 compounds, including both natural and synthetic semiochemical analogs, demonstrated sufficient bioactivity against *C. lectularius* at 24 h and a screening concentration of 1 % to be considered for continued repellent formulation development (Table 8.3). Methyl trans-4-oxo-2-pentenoate and 1-furan-2-yl-2-methylbutan-1-one provided 100 % repellence (Table 8.3) followed by (E)-1-hydroxyoct-2-en-4-one, 6,10-dimethyl-5,9-undecadien-2-one, furfuryl propionate, 2-butyrylfuran, 1-(furan-2-yl)-pentan-1-ol, and (E)-3-methylhept-3-ene-2,5-dione. Furfuryl propionate has been formulated as a fast-acting aerosol (Table 8.5) and as slow-release beads (Table 8.6). These products are currently under review by the joint US Environmental Protection Agency (US EPA) and Health Canada’s Pest Management Regulatory Agency (PMRA). Potential target markets for these products include commercial pest control operators, first responders who are often required to enter infested locations, the hospitality industry (e.g., hotels, cruise lines), and travelers.

Table 8.3 Bed bug repellents screened in the laboratory at 1 % $N = 30$ (three replicates of ten insects)

Compounds	Repellence (%)	Compounds	Repellence (%)
Methyl trans-4-oxo-2-pentenoate	100	1-(Furan-2-yl)-propan-1-ol	79
1-Furan-2-yl-2-methylbutan-1-one	100	6,6-Diethoxyhex-4-yn-3-one	78
(*E*)-1-Hydroxyoct-2-en-4-one	98	(*E*)-3-Methylhept-3-ene-2,5-dione	76
6,10-Dimethyl-5,9-undecadien-2-one	98	(*E*)-Oct-4-ene-3,6-dione	74
Furfuryl propionate	94	(*E*)-1-(Furan-2-yl)pent-1-en-3-one	73
2-Butyrylfuran	92	Ethyl 2-furoate	73
1-(Furan-2-yl)-pentan-1-ol	92	1-(Furan-2-yl)-ethanol	71
(*E*)-3-Methylhept-3-ene-2,5-dione	90	Ethyl 3-methyl-4-oxocrotonate	72

Table 8.4 Bed bug attractants screened in the laboratory at 1 %

Compounds	Attractance (%)
(*E*)-6-Hydroxyhex-4-en-3-one	100
Allyl propionate	96
3-Hexanone	85
3-Nonanone	100
Vinyl propionate	92
N-Methylpropionamide	100
Methyl-4-oxobutanoate	100
3-Pentanone	90

$N = 30$ (three replicates of ten insects)

One lead compound, furfuryl propionate, demonstrated consistent repellence for over 24 h (Fig. 8.2) and has been developed into a rapid-release aerosol formulation and slow-release beads. Repellent effects of the formulation and beads are shown in Tables 8.5 and 8.6, respectively.

8.2.2.3 Repellent Effects of Compounds and an Aerosol Formulation in Glass Arenas

Glass box arenas (Fig. 8.3) consist of rectangular containers (23.5 × 18.5 × 7.0 cm) with lids modified to fit a mesh screen allowing air movement between the box interior and exterior. Test solutions (control versus treated) were applied onto pieces of cloth (10 × 10 cm) placed at opposite ends of the box arena. The position of test subjects was monitored at specific time intervals (1, 2, 4, 6, 8, 12, and 24 h) after initial introduction and repellence were determined for each treatment.

Table 8.5 Repellent effect of furfuryl propionate aerosol formulation in a glass box arena

	Number of bed bugs		
Time (h)	Control	Treated	Repellence (%)
1	26	0	100
2	27	0	100
4	24	0	100
6	28	0	100
8	29	0	100
12	29	0	100
24	23	5	82.1

N = 30 (three replicates of ten insects); control = isopropyl alcohol (IPA); treated = furfuryl propionate (5 %)

Only individuals that are in close contact with the treatment or control substrates were recorded. Bed bugs elsewhere in the arena were not included in repellence calculations

Table 8.6 Repellent effect of furfuryl propionate-treated beads in cardboard box arena

	Number of bed bugs		
Time (h)	Control box	Treated box	Repellence (%)
24	20	6	76.9
48	21	7	75.0
72	19	9	67.8
96	21	6	77.8
120	21	6	77.8
Average	20.4	6.8	75.0

N = 30 (three replicates of ten insects); control box = untreated beads; treated box = beads treated with furfuryl propionate (25 %)

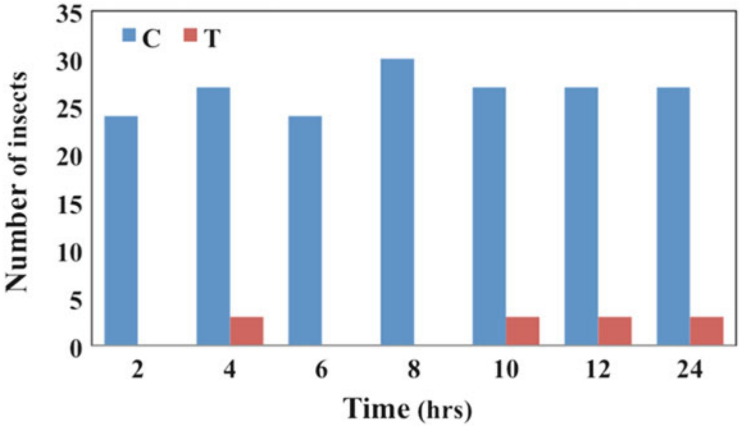

Fig. 8.2 Response of bed bugs to furfuryl propionate (2.5 %) at different time intervals. *Asterisks* indicate significant differences between the control and treated groups for that time period (Tukey's test; $p < 0.05$). Control was isopropyl alcohol (100 %); *N* = 30 (three replicates of ten insects)

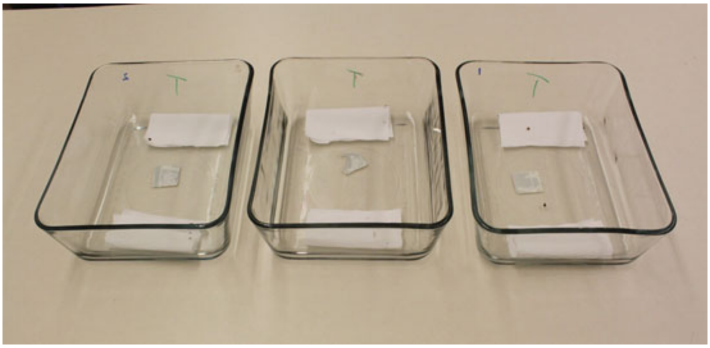

Fig. 8.3 Glass box arenas

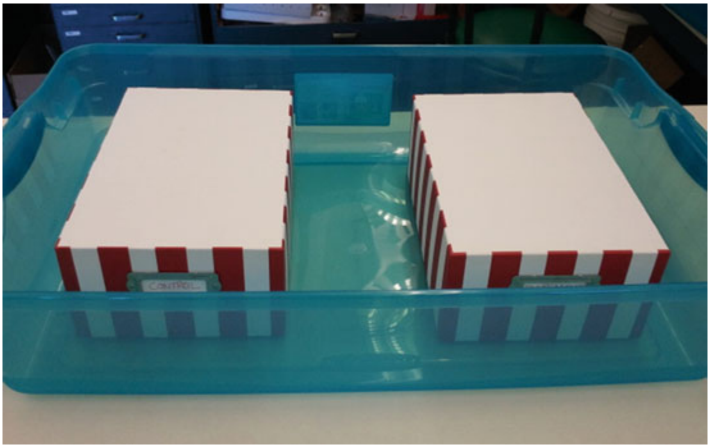

Fig. 8.4 Cardboard box arena

8.2.2.4 Repellent Effects of Beads in Cardboard Boxes

Beads were tested in a cardboard box (suitcase mimic) (Fig. 8.4) arena (28.2 × 19.3 × 11.0 cm). Control/treated beads were introduced into glass Petri dishes (5 cm diameter), placed on each side of a plastic container. Cardboard boxes were inverted over the Petri dishes containing the beads, one for the control and one for the treated beads. Following 24 h of saturation, ten bed bugs were introduced into the center of the plastic container and were allowed to crawl freely under the cardboard boxes. The position of the insects was monitored after 24, 48, 72, 96, and 120 h. Readings were taken by flipping the control box only using the formula: number of bed bugs on the treated beads = number of bed bugs introduced − number of bed bugs on the control and outside the boxes.

Overall, almost 50 % of the compounds demonstrated good activity at a concentration of 1 % against nymphs and adult bed bugs as repellents; 15 compounds consistently produced over 80 % repellence, generally meeting regulatory performance standards for pest control. The most potent compounds produced an average of 100 % repellence.

8.2.2.5 Traps Based on Aggregation Pheromone

Aggregation pheromones are responsible for the formation of conspecific groups of mixed age and sex. The study of aggregation pheromones is the area of bed bug chemical ecology currently attracting the most interest from the scientific community (Weeks et al. 2010; Siljander et al. 2007, 2008; Olson et al. 2009; Gries et al. 2015). The importance of using an aggregation pheromone for monitoring or control purposes is based on the premise that it will be effective against all members of the colony regardless of sex and developmental stage. Recent studies have demonstrated that aggregation of bed bugs is mediated by a combination of airborne (Siljander et al. 2008) and contact pheromones (Siljander et al. 2007; Olson et al. 2009). An airborne aggregation pheromone (Table 8.2) composed of several short-chain aldehydes and monoterpenes occurring in the exoskeleton of immature bed bugs has recently been shown to stimulate aggregation of adult and immature bed bug in harborages (Siljander et al. 2008). Ten compounds (nonanal, decanal, (*E*)-2-hexenal, (*E*)-2-octenal, (2*E*,4*E*)-octadienal, benzaldehyde, (+)- and (−)-limonene, sulcatone, benzyl alcohol) proved to be essential components of the *C. lectularius* airborne aggregation pheromone (Siljander et al. 2008). According to Gries et al., (60) essential attractive volatile pheromone components present in bed bug feces consist of a blend of sulfide compounds (dimethyl disulfide and dimethyl trisulfide), (*E*)-2-hexenal, (*E*)-2-octenal, and 2-hexanone. Although (*E*)-2-hexenal and (*E*)-2-octenal (Levinson and Bar Ilan 1971, 1974; Siljander et al. 2008) have been previously reported as alarm and aggregation pheromone components, the two sulfides and 2-hexanone represent new volatile pheromone components.

Some of the natural constituents of the aggregation pheromone also function as an alarm pheromone at higher concentrations that could be useful as a repellent (Siljander et al. 2008). (*E*)-2-Hexenal and (*E*)-2-octenal were the most abundant compounds associated with aggregation in the headspace collections from bed bug colonies (Siljander et al. 2008). Both of these compounds serve as alarm pheromones at higher concentrations elicited by mechanical disturbance or agitation (Siljander et al. 2008).

8.2.2.6 Traps Based on Heat, Carbon Dioxide, or Chemical Lures

Traps that claim to attract bed bugs currently on the market use either heat (Kells and Goblirsch 2011; Puckett et al. 2013), chemical lures, or both (Anderson et al. 2009). Heated traps mimic a human host to attract hungry bed bugs by producing heat a few degrees higher than the ambient temperature (Moore and Miller 2009). Chemical-baited devices often rely on the use of attractant semiochemicals to lure bed bugs from their refuges and into a trap. Carbon dioxide and heat have been proven to be the two most effective attractants used for bed bug monitoring (Puckett et al. 2013; Singh et al. 2012). Another study also suggests a combination of chemical lure (i.e., 1-octen-3-ol, spearmint oil, and coriander Egyptian oil) and CO_2 to design effective bed bug monitors (Wang et al. 2009b).

Several traps also use a combination of chemical lures, CO_2, and heat to attract bed bugs. A pitfall trap using a combination of heat (37.2–42.2 °C) and kairomones, including a gel lure, impregnated with propionic acid, butyric acid, valeric acid, (RS)-1-octen-3-ol, and L-lactic acid, and CO_2 (Anderson et al. 2009) captured more bed bugs than other traps in a vacant apartment (Table 8.2).

Two commercial monitors, CDC3000 (Cimex Science LLC, Portland, OR) and NightWatch (BioSensory Inc., Putnam, CT), that became available in early 2009 also use a combination of CO_2, heat, and a chemical lure to attract bed bugs. These traps were set up in occupied apartments along with a dry ice trap (Wang et al. 2011). The dry ice trap captured more bed bugs than CDC3000, which in turn was more active than NightWatch. In lightly infested apartments, the ClimbUp Insect Interceptor, a passive monitor without any attractant (operated for 7 days), trapped a similar number of bed bugs as the dry ice trap (operated for 1 day) and trapped more bed bugs than CDC3000 and NightWatch (operated for 1 day). The Interceptor also was more effective than visual inspections in detecting the presence of small numbers of bed bugs (Wang et al. 2011). The chemicals that make up the seven-component blend of CDC3000 have yet to be disclosed.

First Response Bed Bug Monitor (SpringStar Inc., Woodinville, WA, USA), which uses a combination of CO_2, heat, and a synthetic kairomone lure to attract bed bugs, was used to sample bed bugs from two established bed bug populations (Schaafsma et al. 2012). The number of first-instar nymphs caught in the trap was significantly higher than reported in previous studies employing different sampling methods. Another device patented by Siljander et al. (2008) is based on the release of a cocktail of bed bug aggregation pheromones and infrared radiation (Table 8.2).

These devices have several limitations including their high cost and lack of accessibility to the general public. Some of them are no longer available. Moreover, the use of CO_2 in traps is impractical for routine surveillance. Although a number of traps are commercially available, there are few scientific studies that have tested the efficacy of these devices.

Some studies (Anderson et al. 2009; Wang et al. 2011) have demonstrated the potential for trapping as a viable alternative to visual inspections in confirming the presence and size of a bed bug infestation. Wang et al. (2011) showed that certain traps could detect up to 100 % of infestations that have been previously identified by visual inspection.

The potential use of bed bug semiochemicals in monitoring and control of bed bugs has been reviewed (Weeks et al. 2010). Early detection of a bed bug infestation is very important because the larger the infestation, the more difficult eradication will be. Current routine monitoring is limited to visual inspections. Visual inspections are not only labor and time consuming but often seem to miss a large number of bed bugs (Wang et al. 2010).

Trained dogs have been used to detect bed bugs and identify active and inactive refuges (Weeks et al. 2010; Pfiester et al. 2008). This technique may be quicker and more effective than visual inspection, but requires proper training of both the dog and the handler (Fong et al. 2013). Canine inspections are costly (require ∼ USD 900–1500 for inspecting a nursing home, apartment building, or a hotel) (Weeks et al. 2010; Miller 2007), and their performance is often unsatisfactory (Wang et al. 2011). More research is needed to determine factors responsible for canine detection and establishing standard procedures to evaluate the reliability of canine detection services (Wang et al. 2011).

Bed bug monitors are valuable tools in bed bug management. Although ClimbUp Insect Interceptor, dry ice trap, and NightWatch are the most effective monitors known at the present time, none of them provides 100 % (Wang et al. 2011) detection. Moreover, there are no effective commercial products available that are suitable for wide-scale routine surveillance (Weeks et al. 2010).

Therefore, a trap baited with attractive semiochemicals could be effectively used for monitoring bed bug infestations, followed by timely insecticide applications. Additionally, numbers of insects caught in the traps could be helpful in providing information about the geographic distribution of bed bugs (Weeks et al. 2010).

8.2.2.7 Screening Bed Bug Attractants in the Laboratory

We have screened close to 50 semiochemical analogs (Gilbert 2014) for attractant effects with bed bugs in the laboratory. Of this number, at least eight compounds (3-nonanone, 3-hexanone, 3-pentanone, *N*-methylpropionamide, methyl-4-oxobutanoate, (*E*)-6-hydroxyhex-4-en-3-one, vinyl propionate, and allyl propionate) showed sufficient performance (85–100 % attractance) to be considered for continued attractant formulation development (Table 8.4) and could offer the potential for a "push-pull" type of pest management system for bed bugs based on semiochemicals.

Attractant effects were determined in a custom glass Y-olfactometer (Fig. 8.5; length of each arm = 15.3 cm, length of the ally from point of introduction to the choice point = 13.5 cm, diameter of the tubes = 2 cm) at the University of British Columbia, Vancouver, BC, Canada. Each bioassay consisted of at least five test concentrations, each with 30 insects. Bioassays were replicated three times over subsequent days. Glass containers and the olfactometer were washed thoroughly with soap and warm water, baked, and cleaned with acetone before each use.

Attractant compounds offer the potential to produce baited traps for bed bug monitoring. Such monitoring traps could be used in hotels, libraries, hospitals, and other locations that are sensitive to infestation. Traps could be used in residences both to verify the presence of a suspected infestation and to assess the degree of bed bug eradication success. One great advantage of using attractants in monitoring traps is that they are not considered pest control products, so they do not require pesticide registrations in the USA and Canada.

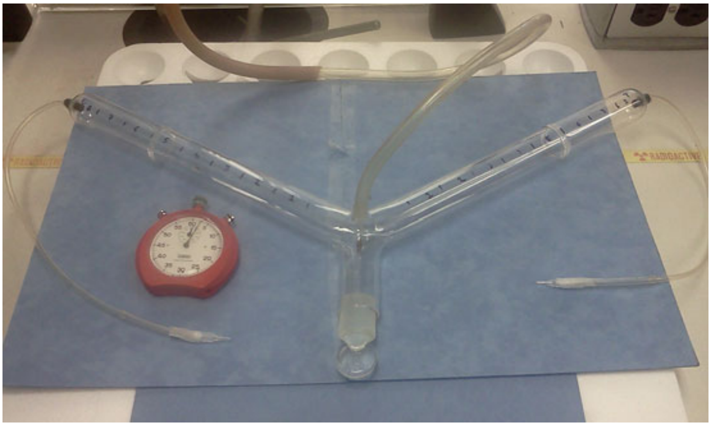

Fig. 8.5 Glass olfactometer

8.2.3 *Role of Biopesticides in Bed Bug Management*

Although a number of products are available in the market, they are rarely adopted by pest management practitioners, due to the lack of scientific data supporting claims of control to date. Nine commonly available biopesticides along with two synthetic insecticides were tested against bed bugs by researchers at Rutgers University. Most of the biopesticides tested failed to satisfactorily control bed bugs through direct spraying. Of the nine products tested, Bed Bug Patrol® (Nature's Innovation Inc., Buford, GA) and EcoRaider® (Reneotech Inc., North Bergen, NJ) showed some promise, but at a much slower speed than the synthetic insecticides tested for comparison (Singh et al. 2013).

Dusts have been used to ward off insects from grain storage for centuries, including "plant ash, lime, dolomite, certain types of soil, and diatomaceous earth (DE) or Kieselguhr" (Hill 1986). Of these, diatomaceous earth in particular has seen a revival as a nontoxic (when in amorphous form) residual pesticide for bed bug abatement. Desiccant dusts are among the oldest forms of insect control agents and are considered effective as long as the insects walk on them (Benoit et al. 2009). Diatomaceous earth chafes and abrades the waxy outer layer of the insect epicuticle resulting in death of the insect due to desiccation (Potter et al. 2013b).

Convincing reports of DE's effectiveness against bed bugs as a nontoxic, eco-friendly alternative include both laboratory (Benoit et al. 2009; Potter et al. 2013b; Akhtar and Isman 2013, 2016; Doggett et al. 2008; Romero et al. 2009b; Anderson and Cowles 2012) and field studies (Wang et al. 2009a). Insects exposed to diatomaceous earth may take several days to die (Benoit et al. 2009); therefore, there is a need to search for new methods to increase the efficacy of diatomaceous earth and decrease killing time. One study aiming to increase the efficacy of desiccant dusts and silica gels for bed bug control involved their application in combination with alarm pheromone components. When (*E*)-2-hexenal and (*E*)-2-octenal were applied either singly or as a blend, in combination with Dri-die (silica aerogel, Fairfield American Corp., Frenchtown, NJ), water loss increased twofold and threefold, respectively, resulting in decreased survival time of first-instar nymphs from 4 days to 1 day (Benoit et al. 2009). A mixture of DE and the pheromone blend demonstrated a 50 % increase in water loss over controls and a decreased survival time from 4 to 2 days in first-instar nymphs. Mixture of the pheromone blend and desiccant dust was more effective than either component alone. Presumably, the addition of alarm pheromone enhanced crawling activity, thereby promoting cuticular damage that increases water loss. While these results are promising, field trials are necessary to determine whether the additive effect of the pheromone is maintained in a natural situation.

Romero et al. (2009a, b) reported high levels of mortality with some commercially available dusts against pyrethroid-resistant bed bug strains. They evaluated five different dusts, two pyrethroid-based dusts, DeltaDust® (0.05 % deltamethrin, Bayer Environmental Science, Montvale, NJ)) and Tempo 1 % Dust® (1 % cyfluthrin, Bayer Environmental Science, Montvale, NJ), and three desiccant dusts, Drione® (1 % pyrethrins, 10 % piperonyl butoxide, 40 % amorphous silica gel, Bayer Environmental Science, Montvale, NJ), MotherEarth D® (100 % diatomaceous earth, BASF), and NIC 325® (99.5 % limestone, ACM – Texas, Loveland, Colorado) against four different populations of bed bugs. Tempo® caused 100 % mortality of bed bugs in all four populations (two highly resistant from Cincinnati and New York, one moderately resistant from Los Angeles, and one susceptible population from New Jersey) within 24 h. Drione also killed 100 % of all the populations, but it took 72 h of continuous exposure, for the two resistant populations from Cincinnati and New York. DeltaDust® caused significant mortality (>90 %) only after 1 week of exposure (Romero 2011).

We have demonstrated strong residual effects of a specific diatomaceous earth (DX13™, DE Laboratories Inc., Vancouver, Canada) dust and an aerosol formulation thereof in the laboratory (Akhtar and Isman 2016) as a reduced-risk bed bug management strategy. The residual effect of DX13™-aerosol persisted for 21 days. The LT_{50} value (Fig. 8.6) increased from 9.1 h for freshly applied DE aerosol to 46.1 h for aerosol aged for 21 days in Petri dishes at an average dosage of 45.8 g m-2. Mortality of the bed bugs in the Petri dishes was >97 % at 72 h for DX13™-aerosol residue aged for 0 h and 14 days. Mortality was 81 % at 72 h for DX13™ aerosol residue aged for 21 days (Akhtar and Isman 2016).

Desiccant dusts, with their physical mode of action and long residual activity, appear to be superior to sprayable pyrethroid products for killing bed bugs (Anderson and Cowles 2012). Comparison of Mother Earth® DE-treated apartments with chlorfenapyr-treated apartments after 10 weeks showed an average 97.6 % bed bug reduction in DE-treated apartments versus 89.7 % reduction in chlorfenapyr-treated ones (Quarles 2015). Moreover, the dust IPM program was less expensive ($463/apartment) than the spray IPM program ($482/apartment) (Quarles 2015).

We also have demonstrated secondary and tertiary mortality of bed bugs through horizontal transfer of DX13™ from exposed to unexposed bed bugs (Akhtar and

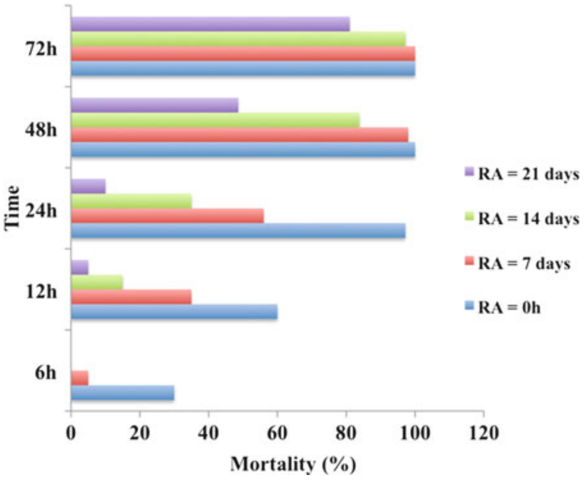

Fig. 8.6 Residual effects of DX13™-aerosol at an average dosage of 45.8 g m-2 (Akhtar and Isman 2016). $N = 5$ replicates of seven to eight insects. Bed bugs were introduced into Petri dishes with residual aging time (RA) of 0 h, 7, 14, and 21 days; mortality was assessed at different time intervals after introduction of insects to treated/control Petri dishes. There was no mortality in the control group

Isman 2013, 2016). Lethal concentrations causing 50 % mortality (LC_{50}) values varied from 24.4 mg of DX13™ dust at 48 h to 5.1 mg of dust at 216 h when a single exposed bed bug was placed with five unexposed bed bugs. Time to kill 50 % of bed bug (LT_{50}) values varied from 1.8 days to 8.4 days when a “donor” bed bug exposed to 20 and 5 mg of DX13™ dust, respectively, was placed with five “recipient” bed bugs (Akhtar and Isman 2016). This result is important because bed bugs live in hard-to-reach places (e.g., cracks, crevices, picture frames, books, furniture), and as such the close interactions between the members of the colony can be exploited for delivery and dissemination for designing effective control strategies.

Pest control operators have been marketing diatomaceous earth as a nontoxic, eco-friendly alternative for years. It is also recommended by government and academic institutions as part of a “Comprehensive integrated bed-bug management program” (Potter et al. 2013b).

8.2.4 Role of Microbials in the Management of Bed Bugs

Fungal species including *Beauveria bassiana* and *Metarhizium anisopliae* also have been used (Table 8.7) to control blood-feeding arthropods (Darbro et al. 2011; Fernandez et al. 2011; Pedrini et al. 2009). Barbarin et al. (2012) evaluated the efficacy of *B. bassiana* as a residual biopesticide against the common bed bug in laboratory conditions. *Beauveria bassiana* (I93-825) was highly virulent to bed bugs, causing rapid mortality (3–5 days) following short-term exposure to spray residues regardless of feeding status, sex, strain, or developmental stage of bed bugs. Barbarin et al. (2012) also evaluated autodissemination of conidia as a means to spread infection among bed bug populations in untreated, inaccessible areas. With respect to test substrates, jersey knit cotton was a better substrate for conidial transfer than paper, probably due to the relatively contoured surface resulting in more conidia coming into contact with the insect cuticle. These results demonstrate that choice of substrate is important in both bioassay design and end product development.

Ulrich et al. (2014) exposed bed bugs to conidia of the entomopathogenic fungus, *Metarhizium anisopliae*, through feeding, aerosol spray, or contact with a treated surface. Mortality was high through feeding, but humidity dependent in other methods of application in laboratory bioassays. Based on the results, they concluded that *M. anisopliae* was a poor pathogen for use in control of bed bugs, particularly at the relative humidity that would likely be encountered under field conditions.

8.2.5 Role of Juvenile Hormone in the Management of Bed Bugs

Naylor et al. (2008) evaluated the effect of the juvenile hormone analog (S)-methoprene (Table 8.7) on adult and nymphal stages of *C. lectularius*. Exposure of nymphs to technical grade (S)-methoprene at a range of doses resulted in an incomplete eclosion, uneven cuticle formation, prolapses of the gut through the dorsal abdominal wall, and formation of supernumerary nymphs. The immature stages could not develop to fertile adults. Response was dose dependent and no normal adults were produced at the highest dose (30 mg/m^2). (S)-Methoprene was as effective against the pyrethroid- and carbamate-resistant strain as it was against the susceptible strain, suggesting that there is currently little or no field resistance or cross-resistance to this compound.

8.2.6 Nonchemical Tools

Although nonchemical tools, such as temperature treatments (e.g., steam and dry ice), mattress encasement, sanitation, and vacuuming, are available, only the spraying of insecticides provides long-term control and prevents against reinfestation of bed bugs (Doggett et al. 2004). Several studies have demonstrated the use of temperature to control bed bugs (Table 8.7). The lethal temperatures required to kill 99 % of adult bed bugs (LT_{99}) and their eggs were 48.3 °C and 54.8 °C, respectively; time to kill 99 % of adult bed bugs exposed to 45 °C was 94.8 min; eggs survived for 7 h at 45 °C but only 71.5 min at 48 °C (Kells and Goblirsch 2011). Puckett et al. (2013) exposed all stages of bed bugs to three steam treatment exposure periods and demonstrated that mortality of bed bug eggs was 100 % (regardless of duration of exposure) and that of nymphs and adults ranged from 88 % to 94 %. Rukke et al. (2015) exposed adult bed bugs to sublethal temperatures 34.0 °C, 35.5 °C, 37.0 °C, 38.5 °C, or 40.0 °C for 3, 6, or 9 days. The two uppermost

Table 8.7 Response of bed bugs to natural products ± insecticide/physical control

Source	Effect	Additional note	References
(S)-Methoprene (juvenile hormone analog)	Failure of immature stages to develop to fertile adults	Active against both resistant and susceptible strains	Naylor et al. (2008)
Metarhizium anisopliae	Dose-dependent mortality through feeding	Mortality was 100 % through feeding and humidity related through spraying and contact with the treated surface	Ulrich et al. (2014)
Temperature lethal/sublethal	Mortality sterilization	Strong correlation between mortality and temperature as well as different stages of bed bugs	Kells and Goblirsch (2011); Puckett et al. (2013)
Three steam treatment exposure periods using a portable device	Controlling localized infestations	100 % mortality – eggs Steam could be used as a practical component of IPM to manage bed bugs	Rukke et al. (2015)
Beauveria bassiana	Mortality, horizontal transfer of fungal spores	Bed bugs were exposed to paper and cotton jerseys treated with spore formulation of *B. bassiana* for an hour died within 5 days	Barbarin et al. (2012)
Desiccant dust + (*E*)-2-hexenal or (*E*)-2-octenal or their blend	Enhanced efficacy of dust	Increased movement of bugs enhanced exposure of bugs to desiccant dust leading to mortality and water loss	Benoit et al. (2009)
Desiccant dust and aerosol formulation	Mortality, horizontal transfer	Contact and residual effects; DE dust was transferred from infested bed bugs to uninfested bed bugs and caused mortality	Akhtar and Isman (2013, 2016)
Insecticidal dusts	Enhanced efficacy of dust	Dust products containing an insecticide had long residual activity and were more superior to sprayable pyrethroid products for killing bed bugs	Anderson and Cowles (2012)
Various laundering methods	Mortality	Washing clothes at 60 °C, drying at >41 °C, and freezing at −17 °C killed all stages of bed bugs	Naylor and Boase (2010)
Combination of chemical and nonchemical methods	Mortality/controlling infestation	Washing and cleaning/throwing away infested belongings combined with several insecticide applications	Fuentes et al. (2010)

temperatures induced 100 % mortality within 9 and 2 days, respectively, whereas 34.0 °C had no observable effect. The intermediate temperatures interacted with time to induce a limited level of mortality but had distinct effects on fecundity in terms of decreased number of eggs produced and hatching success (Rukke et al. 2015).

Comparison of various laundering methods (Table 8.7) to disinfect clothing infested with bed bugs demonstrated that washing at 60 °C, tumble drying for at least 30 min on the hot cycle (>40 °C), dry cleaning with perchloroethylene, or freezing for at >2 h at −17 °C killed all stages of bed bugs (Naylor et al. 2008). However, soaking items in detergent-free water for 24 h was sufficient to kill bed bug adults and nymphs but not the eggs (Naylor et al. 2008).

The concern over the itchy bites of bed bugs followed by development of secondary infections has led to the development of a new sterilization system (AsepticSure®, Medizone International Inc., Sausalito, CA) in hospitals that can kill the highly drug-resistant bacteria as well as the bed bugs (Wjeczner 2013). Medizone International Inc. has already started distributing its new disinfecting technology to hospitals in Canada and is seeking its approval to market it in the USA. Although this system took less than an hour to eradicate 100 % of bacteria, it took 24 h to kill bed bugs and 36 h to kill their eggs (Wjeczner 2013).

A combination of chemical and nonchemical means (washing and cleaning all affected belongings, throwing away infested belongings, and several insecticide applications) were required to control bed bug infestation in three homes (Table 8.7) in Valencia (Spain) (Fuentes et al. 2010), occupied by people who have acquired bed bugs during their travel to the UK, Spain, and Sweden prior to the study.

8.3 Future Directions

Evaluations of populations from across the USA and other parts of the world indicate that resistance to pyrethroid insecticides is widespread. This inability to control bed bugs with pyrethroids necessitates development of products with new modes of action, relabeling of existing efficacious products, and greater reliance on alternative tactics such as heat treatment, vacuuming, mattress encasements, or barriers (Romero et al. 2007). Development of delivery systems based on barrier treatments, such as a “bed skirt,” positioned between the harborages and the human host demonstrates potential for effective control (Barbarin et al. 2012).

There will be an increased demand for the development of novel behavior-modifying substances such as effective repellents and attractants based on semiochemicals or other natural products. A trap (or traps) containing a lure based on a natural or synthetic blend of semiochemicals may be one strategy for diverting bugs from human hosts or to partially “trap out” a resident population. Targeting control to homes, rooms, and areas that are infested with bed bugs will reduce insecticide use (Reinhardt and Silva-Jothy 2000).

A further strategy is the stimulo-deterrent diversionary strategy, or "push-pull" strategy, that combines an attractant and a repellent. Yet another strategy is "attract and kill" that combines an attractant with a toxic product. For the development of such pest management systems, it is essential to establish a full understanding of bed bug chemical ecology and behavior.

In conclusion, insecticide-only treatments for bed bugs will likely fail due to resistance and cross-resistance development. The best hope is an IPM program using components such as prevention, monitoring, vacuuming, traps, repellents, heat and steam, fumigation, and use of reduced-risk pesticides such as silica gel, diatomaceous earth, neem, essential oils, and microbials. As diatomaceous earth (DE) has an extremely long residual action and as its mode of action limits the possibility of resistance developing, there is a strong potential for DE dust to be employed as a preventative insecticide, which further enhances the prospect for strong financial returns. A multidisciplinary strategy with several key components including a code of practice for the control of bed bug infestations that defines and promotes best practice for bed bug eradication, development of a policy and procedural guide for accommodation providers, and education of stakeholders should be adopted similar to Australia and other countries (Doggett et al. 2011b). Even with all these options, complete elimination of bed bugs from a structure is very difficult (Quarles 2015). Without the development of new tactics or approaches for bed bug management, further escalation of this pest should be expected.

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