Bed Bug (Heteroptera: Cimicidae) Attraction to Pitfall Traps Baited With Carbon Dioxide, Heat, and Chemical Lure

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ABSTRACT Carbon dioxide (CO_2), heat, and chemical lure (1-octen-3-ol and L-lactic acid) were tested as attractants for bed bugs, *Cimex lectularius* L. (Heteroptera: Cimicidae), by using pitfall traps. Both CO_2 and heat were attractive to bed bugs. CO_2 was significantly more attractive to bed bugs than heat. Traps baited with chemical lure attracted more bed bugs but at a statistically nonsignificant level. In small arena studies (56 by 44 cm), pitfall traps baited with CO_2 or heat trapped 79.8 ± 6.7 and 51.6 ± 0.9% (mean ± SEM) of the bed bugs after 6 h, respectively. Traps baited with CO_2 + heat, CO_2 + chemical lure, or CO_2 + heat + chemical lure captured ≥86.7% of the bed bugs after 6 h, indicating baited pitfall traps were highly effective in attracting and capturing bed bugs from a short distance. In 3.1- by 1.8-m environmental chambers, a pitfall trap baited with CO_2 + heat + chemical lure trapped 57.3 ± 6.4% of the bed bugs overnight. The pitfall trap was further tested in four bed bug-infested apartments to determine its efficacy in detecting light bed bug infestations. Visual inspections found an average of 12.0 ± 5.4 bed bugs per apartment. The bed bugs that were found by visual inspections were hand-removed during inspections. A pitfall trap baited with CO_2 and chemical lure was subsequently placed in each apartment with an average of 15.0 ± 6.4 bed bugs collected per trap by the next morning. We conclude that baited pitfall traps are potentially effective tools for evaluating bed bug control programs and detecting early bed bug infestations.

KEY WORDS *Cimex lectularius*, lure, trap, monitoring

The recent resurgence of the bed bug, *Cimex lectularius* L. (Heteroptera: Cimicidae), in the United States, Canada, Australia, and some European countries triggered strong interests among researchers and the pest control industry to investigate effective bed bug management tactics (Cooper 2006, Gangloff-Kaufmann et al. 2006, Harlan 2006,Doggett 2007). Bed bug infestations often go unnoticed until becoming a serious problem. Once established, they are difficult and expensive to eradicate due to insecticide resistance and lack of effective control tools (Cooper 2006, Romero et al. 2007).

Detecting bed bugs during the early stages of an infestation and confirming the elimination of bed bugs after treatment are critical in effective management of bed bugs and will minimize the long-term management cost. Unfortunately, bed bugs are difficult to locate during visual inspections due to their small size and cryptic behavior. A full inspection of an occupied room usually requires two experienced technicians and hours of labor. Even so, physical inspections are unreliable and often severely underestimate the actual number of bed bugs in apartments (Wang et al. 2009).

Some pest management professionals have experimented with sticky traps as a detection tool for bed bugs and found them to be ineffective when used alone (Cooper 2006). Lang et al. (2007) described a system using a sticky surface and pitfall trap for trapping bed bugs. However, there is no data demonstrating the effectiveness of the concept. Detection dogs were found effective for identifying light bed bug infestations (Pfiester et al. 2008) but can be very expensive and the accuracy of this method is affected by a variety of factors including trainer's experience, dog breed, and environmental factors.

Carbon dioxide (CO_2), heat, and 1-octen-3-ol (octenol) are widely used in commercial traps to attract adult mosquitoes (Kline 2006). Bed bugs are attracted to heat, odor, and CO_2 (Rivnay 1932, Marx 1955, Aboul-Nasr and Erakey 1967). It is also known that both CO_2, octenol, and combinations of several short-chained fatty acids—proprionic, butyric, valeric, and L-lactic acid— have stimulating effect on the blood-sucking bug *Triatoma infestans* (Klug) (Barrozo and Lazzari 2004a,b). Anderson et al. (2009) investigated bed bug response to pitfall traps baited with CO_2 (emitted from a pressurized gas tank), thermal lure, and chemical lure (mixture of five components). Only CO_2 was found to significantly and consistently increase trap catches compared with traps without CO_2 both in laboratory and field experiments. Heat or lure (combination of proprionic acid, butyric acid, valeric,

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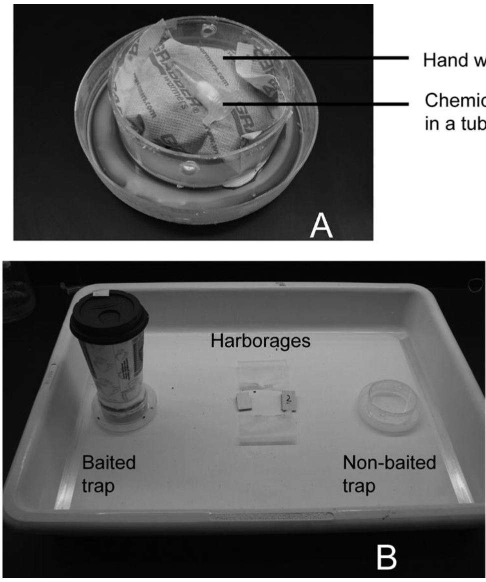

Fig. 1. Experimental setup for evaluating bed bug attraction to baited pitfall traps. (A) Pitfall trap. (B) Experimental arena.

acid, octenol, L-lactic acid) alone did not consistently increase trap catches.

Based on results from Anderson et al. (2009), Biosensory Inc. recently developed a commercial (Nightwatch) bed bug monitor. Around the same time, Cimex Science LLC (Portland, OR) developed CDC3000 bed bug monitor. Both devices use CO_2, heat, and a chemical lure to attract bed bugs. Yet, there are no experimental data demonstrating the effectiveness of these traps and role of chemical and nonchemical lures on trap catches. Each device is estimated to cost several hundred to a thousand dollars.

So far, our knowledge about bed bug response to chemical and nonchemical lure is still very limited. In particular, the relative attractiveness of CO_2, heat, and chemical lure to bed bugs is poorly understood. The objectives of this study were to 1) investigate the attractiveness of various stimuli (CO_2, heat, and chemical lure) to bed bugs and 2) evaluate the utility of an economic bed bug monitor for detecting low levels of bed bug infestations.

Materials and Methods

Insects. Bed bugs (adults and fourth- and fifth-instar nymphs) were collected from infested apartments in Indianapolis, IN. They were maintained in glass jars with folded filter paper as harborages. The bed bugs were not fed during the study period. They were kept at 22–23°C, 24–48% RH, and a photoperiod of 12:12 (L:D) h.

Experiment 1. Bed Bug Attraction to Pitfall Traps Baited with CO_2, Heat, and Chemical Lure. A pitfall trap made of two plastic dishes was designed to evaluate bed bug attractant (Fig. 1A). The small dish size was 6.3 cm in diameter and 2.2 cm in height. The large dish was 8.8 cm in diameter and 1.9 cm in height (Gary Plastics Packing Corp., Bronx, NY). A layer of fluoropolymer resin (DuPont Polymers, Wilmington, DE) was applied to inner walls of the large dish and exterior walls of the small dish to confine bed bugs that fell into the trap. The exterior surface of the large dish was roughened with sand paper to allow bed bugs to climb up. Plastic arenas (55.5 by 43.5 by 7.5 cm [length by width by height]) were used to evaluate bed bug attractant (Fig. 1B). The bottom of each arena was covered with fabric. A layer of fluoropolymer resin was applied to inner walls to prevent bed bugs from escaping. Two pieces of wood and two pieces of folded fabric were placed at center of the arenas as harborages.

Twenty-five bed bugs that were collected from infested apartments 7–8 d before the experiment were released into each arena. Two traps were placed in each arena (Fig. 1B). The bed bugs were confined to the center of each arena under an 11-cm-diameter petri dish for 24 h. After conditioning and at 1 h before the dark cycle, one trap in each arena received one of the following three types of attractants: 1) CO_2, 2) heat, 3) chemical lure (1-octen-3-ol + L-lactic acid) (Fig. 1B). The other trap in each arena was not baited. The petri dish confining the bed bugs was removed. Three arenas were placed in a room and separated by 2–3 m to minimize the confounding effects from the attractants. The experiment was repeated three times over three consecutive days. Each day, a different attractant type was placed in each arena following a Latin square design.

The CO_2 source was dry ice in double cups (a 236-ml foam cup inside a 355-ml insulated paper cup) with a CO_2 release rate of 169 ml/min. CO_2 was released from a small orifice located on the lid of the large cup. The heat source was a mini hand warmer (Grabber Grand Rapids, MI) placed inside the small dish. Its surface temperature from 1 to 5 h after activation was 43.3–48.8°C. Air temperature immediately above the exterior wall of the pitfall trap (without dry ice cup) measured hourly from 1 to 5 h after activation was 23.2–23.3°C. The temperature decreased to 22.9°C at 21 h. The room temperature was 22.4°C during the study period. The air temperature beside traps with dry ice cup might have been negatively affected. The chemical lure consisted of 50 μl of 1-octen-3-ol and 50 μl of L-lactic acid was dispensed on cotton within a 0.7 ml micro centrifuge tube. Both chemicals were purchased from Sigma-Aldrich (St. Louis, MO). The tube lid had a 2-mm-diameter opening to allow for slow release of the chemicals. Both 1-octen-3-ol and L-lactic acid were effective in attracting bed bugs in our preliminary studies. The number of trapped bed bugs was recorded hourly for 6 h and once at 21 h. The percentage of trapped bed bugs was calculated by dividing the number of bugs in the trap by the total number of healthy bugs inside and outside the trap at the end of the experiment. A healthy bed bug was defined as being able to walk normally after gently prodding with forceps.

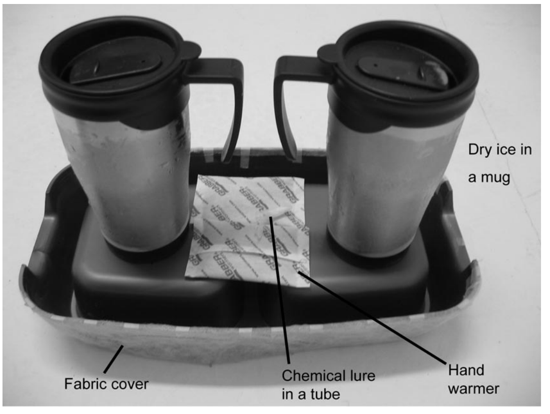

Fig. 2. Baited pitfall trap for monitoring bed bug populations.

Experiment 2. Bed Bug Attraction to Pitfall Traps Baited with Lure Combinations. Four attractant combinations (treatments) were evaluated under similar conditions as experiment 1: 1) heat + CO_2; 2) heat + chemical lure; 3) CO_2 + chemical lure; and 4) heat + CO_2 + chemical lure. The CO_2 release rate was 83 ml/min. This rate was equally effective as that used in experiment 1 in attracting bed bugs from a short distance. The objective of this experiment was to investigate whether combinations of two or three attractants are more effective in attracting bed bugs and which combination is most effective. The four treatments were evaluated in four arenas on the same day. Each treatment was assigned to a different arena and the experiment was repeated four times over four consecutive days following a Latin square design. Each arena contained 30 bed bugs, which were collected from infested apartments 7–19 d before the experiment. They were conditioned for 24 h before treatments were applied.

Experiment 3. Effectiveness of Baited Pitfall Traps for Detecting Bed Bugs in Environmental Chambers. An experimental pitfall trap made from an inverted cat feeding dish (35.5 by 17.5 by 7 cm [length by width by height]; Van Ness Plastics, Clifton, NJ) was evaluated for attracting and trapping bed bugs from long distances (Fig. 2). The trap design was the same as that used by Anderson et al. (2009). A thin layer of talcum powder (Spectrum Chemical Manufacturer Corp., Gardena, CA) was applied to inner surfaces of the inverted cat feeding dish. Two mini-hand warmers, two coffee mugs (390-ml capacity) filled with dry ice, and a 0.7-ml tube of L-lactic acid and octenol (50 μl each, absorbed in cotton) were placed on top of the feeder as baits. The total CO_2 release rate from the two mugs was 498 ml/min (approximating 2–3 times equivalent of an adult human) (Leff and Schumacker 1993). This lasted for 8–9 h. The CO_2 release rate was increased by a factor of five and the heat source was doubled, compared with those in experiment 2. Because of the presence of dry ice, the air temperature above the upper edge of the pitfall trap was 6.9°C lower than the chamber temperature at the onset of the experiment and was never higher than the chamber temperature during the experiment period.

The experiment was conducted in four walk-in chambers (3.05 by 1.83 by 2.67 m [length by width by height]) in a laboratory. A plastic film was taped along the floor-wall conjunction to prevent bed bugs from climbing up the chamber walls. A thin layer of talcum powder was applied to the plastic film for added prevention of escaping. The chambers were kept at 25–27°C, 23–50% RH, and a photoperiod of 12:12 (L:D) h. Six pairs of harborages were placed at equal distances along perimeters of the chamber floor. Each pair of harborages included 3.5- by 3.5-cm folded cardboard and 8- by 8-cm folded white cloth. Thirty bed bugs were evenly released along perimeters of each chamber. These bugs were collected from infested apartments 3 d before the experiment.

After 1 d of acclimation in the chambers, a pitfall trap was placed at the center of each chamber 1 h before the dark cycle. The trap was examined after 21 h. Trapped bed bugs were counted and released back into the chambers. Dead bed bugs were replaced with healthy bed bugs. Each trap was then recharged 1 h before dark cycle. This process was repeated three times over three consecutive days.

Experiment 4. Effectiveness of a Baited Pitfall Trap for Detecting Low Levels of Bed Bug Infestations in Occupied Apartments. Utility of the pitfall traps as described in experiment 3 was evaluated in four bed bug-infested one-bedroom apartments. Each trap was baited with CO_2 and chemical lure. Heat was not provided because adding heat to the CO_2 + chemical lure combination did not significantly improve the trap catches in experiment 2. Three of the apartments were treated with hot steam and/or insecticide spray (0.5% chlorfenapyr) 2–4 wk before this experiment. One apartment was treated with pyrethroids (dust and spray) 1 yr before this study. The apartments were visually inspected for live bed bugs. Bed bugs that were found during inspection were immediately removed. Afterward, a trap was placed in the late afternoon (3:30–4:30 p.m.) beside the infested bed or sofa in each apartment and was examined the next morning. Residents stayed in the apartment, but not necessarily in the same room with the trap. The visual inspections found ≤23 bed bugs in each apartment and they were considered as low levels of infestations.

Experiment 5. Effectiveness of a Baited Pitfall Trap for Detecting Bed Bug in an Unoccupied Apartment. A heavily infested apartment which had ≈500 bed bugs by visual inspection was identified. Most of the bed bugs were found on a sofa in the living room. The bedroom was nearly empty. The senior author of this paper treated the apartment with hot steam and 0.5% chlorfenapyr. The resident discarded the sofa and all other furniture and moved out of the apartment within a week after treatment. At 13 and 21 d after vacancy, a baited trap was placed where the sofa was located. The trap was examined the next morning.

Data Analysis. A paired *t*-test was used for comparing trap catches between baited and nonbaited traps. Analysis of variance (ANOVA) was used to compare

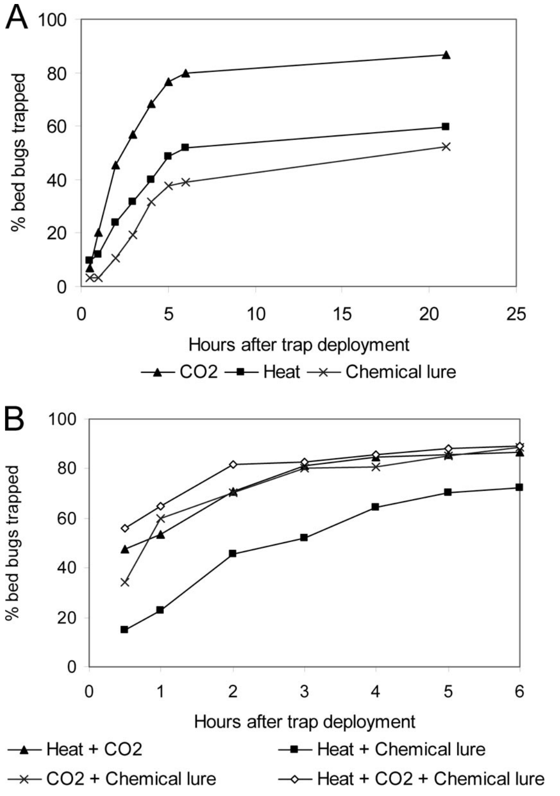

Fig. 3. Percentage (mean ± SEM) of bed bugs attracted to pitfall traps baited with a single attractant (A) and a combination of two or three attractants (B).

percentage of trap catches among different treatments. This was followed by Tukey's honestly significant difference test to separate the means. Percentage of bed bugs trapped in the pitfall traps over time (days) was analyzed using repeated measurement analysis. All analyses were performed using SAS software (SAS Institute 2003).

Results

Bed Bug Attraction to Pitfall Traps Baited with CO_2, Heat, and Chemical Lure. Bed bugs were detected in traps within 30 min of trap deployment. Most of the trap catches occurred within 6 h (Fig. 3A). Traps baited with CO_2, heat, and chemical lure captured 79.8 ± 6.7, 51.6 ± 0.9, and 39.0 ± 5.6% of the bed bugs from the arenas at 6 h, respectively. In contrast, the accompanying nonbaited traps trapped 3.0 ± 3.0, 16.0 ± 5.3, and 31.3 ± 11.7% of the bed bugs, respectively. Paired comparisons show that traps baited with CO_2 or heat captured significantly more bed bugs than nonbaited traps ($P < 0.05$). Chemical lure did not significantly increase trap catches ($t = 0.87$, df = 2, $P = 0.48$). CO_2 was significantly more attractive to bed bugs than heat ($F = 17.2$; df = 2, 6; $P = 0.003$).

In experiment 2 evaluating bed bug attraction to four combinations of attractants, the mean percentages of bed bugs in the nonbaited trap were <2.5%.

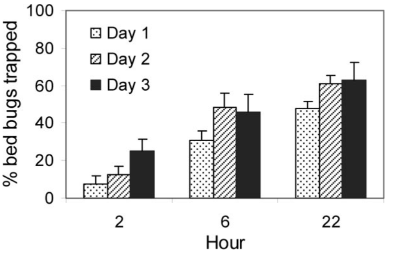

Fig. 4. Daily changes in trap catches (mean ± SEM). Thirty bed bugs were in each walk-in chamber every day.

The mean percentages of bed bugs in traps baited with heat + CO_2, heat + chemical lure, CO_2 + chemical lure, and heat + CO_2 + chemical lure were 86.7 ± 3.7, 72.1 ± 6.6, 88.7 ± 3.5, and 89.0 ± 1.0%, respectively (Fig. 3B). All combinations containing CO_2 were significantly more attractive to bed bugs than that without CO_2 ($P < 0.05$; Tukey's test) and attracted bed bugs much faster than CO_2 alone.

Effectiveness of Baited Pitfall Traps for Detecting Bed Bugs in Environmental Chambers and in Apartments. In the chamber experiment, the mean percentages of bed bugs (average of 3 d and four chambers) captured by baited traps were 15.1 ± 2.5, 41.8 ± 7.4, and 57.3 ± 6.4% at 2, 6, and 22 h after placement, respectively (Fig. 4). There were no significant differences in the trap performances among the 3 days at 2, 6, or 22 h ($P > 0.05$; ANOVA).

In occupied apartments, baited pitfall traps captured bed bugs that were not detected by visual inspections in apartments (Table 1). The mean numbers of bed bugs detected by trapping and by visual inspection were 15.0 ± 6.4 and 12.0 ± 5.4, respectively. In the unoccupied apartment, the baited pitfall trap captured 505 bed bugs (91.1% were nymphs) on 13 d after vacancy and 113 bed bugs on 21 d after vacancy.

Discussion

We have demonstrated that baited pitfall traps can be used as a tool to aid in the detection of bed bugs and can be used to help evaluate effectiveness of bed bug control programs. Pitfall traps baited with CO_2 and chemical lure captured bed bugs that were not discovered by visual inspections in apartments. A visual inspection to identify low levels of bed bug infestation is a very time consuming and difficult task. In contrast,

Table 1. Effectiveness of pitfall traps baited with chemical lure and CO_2 for detecting bed bugs in apartments

Apartment	Trap count	Visual count	Trap location	Human host location
1	5	6	Living room	Bed room
2	33	0	Bed room	Living room
3	7	19	Living room	Living room
4	17	23	Bed room	Bed room

One trap was placed in each apartment after visual inspection and hand-removal of bed bugs found by visual inspection.

preparing and examining baited pitfall traps require little effort and are much more efficient than visual inspection, especially in multiunit buildings where many apartments need to be inspected in a short period of time.

The small pitfall traps made of plastic dishes provide an efficient method to quantify the attractiveness of nonchemical and chemical lures to bed bugs. Among the three attractants (CO_2, heat, and chemical lure) tested, we found that CO_2 was by far the most effective in attracting bed bugs, supporting the conclusion by Anderson et al. (2009). Additionally, we found that heat alone was effective in attracting bed bugs. Chemical lure increased trap catches in two of the three replicates at 6 h and in all replicates at 21 h. However, the differences were small. This result was consistent with our preliminary tests showing weak attractiveness of chemical lures. The mixture of L-lactic acid and octenol might have negatively affected each other's release rate. The synergistic effect between CO_2 and other lures was not analyzed statistically because the CO_2 rate was not uniform in the two experiments. Yet, adding heat or chemical lure significantly improved the rate and speed of trap capture.

In environmental chamber studies, we initially tested the small pitfall traps for 1 d. Only 18.1 ± 7.3% bed bugs were captured. We then used a much larger trap design, higher CO_2 release rate, and doubled the heat source in the chamber experiment to compensate for the much larger experimental area compared with experiment 2. Even so, a much lower percentage of bed bugs was trapped compared with experiment 2 (57.3 versus 89.0), suggesting that both distance between bed bugs and the trap and lure release rate or size are important factors affecting trap efficacy. Using exactly the same inverted cat feeder, Anderson et al. (2009) reported 58.8–77.5% of released adult bed bugs were captured overnight by a pitfall trap baited with CO_2 (released from a pressurized tank) in a 1.83-m^2 arena. The very similar capture rates verified that both CO_2 delivery methods were effective in attracting and capturing bed bugs. This is important because dry ice is much more available and affordable than pressurized CO_2 tanks or cartridges. The hand warmers were not able to generate enough heat to raise the air temperature above the trap when dry ice cups were present. Therefore, the effect of the hand warmers on trap catches was dubious in this design and needs to be evaluated.

The 2-h trap catch in experiment 3 ranged from 0 to 20% on day 1 and from 10.5 to 36.4% on day 3. Although the mean number on day 3 was much larger than that on day 1 (Fig. 4), there were no detectable significant differences. During the 3-d experiment, an average 27.2 ± 2.9% bed bugs died each day, indicating that many of the tested insects might have suffered from hunger, contact with talcum powder inside the trap, or injury. Yet, the trap performance was consistent over the 3-d experiment period, validating the reliability of baited pitfall traps for estimating bed bug populations.

Anderson et al. (2009) documented the performance of baited pitfall traps both in occupied and unoccupied apartments but the baseline bed bug population levels were not determined. Thus, the relative efficacy of the trap was not clear. Our study in apartments with known bed bug population levels demonstrated that pitfall traps were able to detect low level bed bug infestations, even in the presence of a human host. We were not able to control the relative location of the trap and the human host (e.g., in the same room or in different rooms). All traps caught bed bugs that were not found by visual inspections, showing that small numbers of bed bugs were still present, and competition from human host did not inhibit pitfall traps from detecting low numbers of bed bugs.

Bed bugs can live without a human host for an extended period. In unoccupied apartments where bed bugs are still present, the effectiveness of baited pitfall traps may be higher because bed bugs are hungrier than those with access to human host. The single test in a vacant apartment showed that in heavily infested apartments 1) visual inspections could seriously underestimate the bed bug numbers, 2) large numbers of bed bugs were not on the furniture and survived the chemical and nonchemical treatment, and 3) baited-pitfall traps were helpful in monitoring effectiveness of bed bug treatments. From our observations, bed bugs frequently travel from infested apartments to the hallways in a multiunit apartment building (C.W. et al. unpublished data). It is logical to infer that bed bugs are more likely to disperse into neighboring apartments through hallways when their host is no longer present. Therefore, using baited traps in unoccupied infested apartments may reduce the risk of bed bug dispersal between adjacent units in multiunit dwellings.

Basic research into bed bug's biology, behavior, and ecology is required for optimizing the trap design and using traps in a way that maximizes efficacy. Considering the current difficulties in bed bug detection and control (Cooper 2006, Romero et al. 2007, Potter 2008), and the continuing spread of bed bug infestations in the United States (Gangloff-Kaufmann et al. 2006), an effective and reliable bed bug monitoring tool will probably play a pivotal role in safeguarding human health, reducing insecticide applications, and minimizing economic losses associated with bed bug infestations.

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