Final Report

Research Proposal to the California Structural Pest Control Board for Structural Pest Control Research #10-C0074-DPR

Evaluations of Monitors for the Bed Bug, *Cimex lectularius* Linnaeus

Vernard R. Lewis[1,2], Sara Moore[2], Robin Tabuchi[2]

[1]Principle Investigator

[2]University of California, Berkeley

Department of Environmental Science, Policy and Management

1301 South 46th Street, Bldg. 478

Richmond, CA 94804

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[2] The statements, figures, and tables contained in this report fulfill the reporting requirements for the contract and have not undergone peer review. Copies of all final peer-reviewed and accepted manuscripts from this report will be forwarded to the Structural Pest Control Board at a later date.

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EXECUTIVE SUMMARY

Laboratory and simulated-field tests were conducted on passive (non-attractive) and active (attractive) monitors to evaluate their performance in capturing adult bed bugs. For laboratory investigations, five monitors were included, three passive and two active. The methods of evaluation included the construction of three 2 x 0.3 x 0.3 m test arenas made of wood and plastic. Five males and five females were placed into one end with a small cardboard harborage, and the other end of the same arena, one of five monitors was placed. All assignments of monitors were randomized. The monitors, harborage, and bed bugs were left overnight. The following day, the number of bed bugs captured in monitors, remaining in harborage source, and those found on the floor of arenas were counted. Three replications for each of five monitors and untreated check were conducted. Both fed and starved conditions for bed bugs were included in treatment replications. In the laboratory tests we did not find any significant differences among monitors in the percentage of bed bugs captured with respect to gender or feeding condition. Bed bug foraging was considerable, however, and they were found away from the harborage source 66% of time. For simulated-field tests, a large 154 m^3 test structure was renovated to accommodate live, foraging bed bugs. Within this structure we used four custom-built arenas that consisted of two 4-ply Douglas fir 2.4 m by 1.2 m by 1.1 cm (lwh) plywood sheets laid along their long axis. Each arena was surrounded by four clear 2.36 m by 0.48 cm by 10.2 cm (lwh) polycarbonate plastic strips. Each plastic strip was secured to the wooden test floor using metal brackets, wood screws, and caulked along all seams. A wagon-wheel design was used inside the wooden arenas that featured a centrally located harborage source and release point that was a 38.1-cm radial distance to positions on the floor to test the attractiveness or non-attractiveness of monitors. These tests also featured a choice comparison for bed bugs that comprised a monitor and furniture items that included a small bed, table, and rug. The parameters tested included monitor type (two passive and one active) and bed bug densities of 10, 50, or 100. Equal numbers of starved males and females were included for all densities tested. All monitor treatment and densities were randomly assigned. All treatments were conducted at ambient

conditions and left over night for bed bugs to move among arenas contents. The following day, counts were made of bed bugs captured by monitors, remaining in harborage source, in or on furniture items, and scattered about the floor. To further insure the completeness of counts, all monitors, harborage sources and furniture items were disassembled and searched for concealed or hidden bed bugs. All arenas and contents were cleaned between replicates. In total, thirty replicates were completed for monitor and density combinations. Results from the simulated-field tests were similar to laboratory investigations, considerable foraging in the test arenas and furniture items; however there were no significant differences in the percentage of bed bug captured among monitors. Bed bugs were found at least 60% of time away from harborage sources, and a significantly higher percentage of males were found in harborage sources compared to females. The conclusions drawn from both laboratory and simulated-field studies are that none of the five monitors tested had any significantly greater ability to capture bed bugs when compared to untreated checks or other furniture items contained in arenas. Additional conclusions reached include: (1) there were no correlations in monitor attractiveness and performance with increase bed bug density, (2) females tended to wander more and farther than males, and (3) population estimates using monitors was at worst 5% of the original number released. The implication of these findings on expected monitor performance under field conditions and estimating infestation size are discussed.

BACKGROUND

For centuries bed bugs *Cimex lectularius* (L.) (Hemiptera, Cimicidae), were widespread within human habitations. Archeological evidence suggests bed bugs first plagued humans living in caves (Usinger 1966, Potter 2011). As people moved from caves into villages and cities, it was an easy transition for bed bugs to establish themselves in their new human surroundings (Potter 2011). Through the millennia, historical accounts of infestations were reported, irrespective of class strata or economic condition (Potter 2011). Bed bug infestations were frequently encountered in many parts of the world, including North America, for several decades into the 1900s (Doggett and Russell 2008, Cooper 2011, Potter 2011).

The decline in bed bug infestations started during World War II was due, in part, to the successful control campaign that included exclusively using DDT (Ebeling 1978, Cooper 2006, Moore and Miller 2006, Doggett and Russell 2008, Zhu et al. 2010, Cooper 2011). This control campaign supported US troops and included barracks, battlefield trenches, military-issued personal equipment, transport equipment and ships, as well as other public facilities throughout the world (Potter 2011). After the war, general improvements in household and personal cleanliness, and widespread use of synthetic insecticides (primarily organochlorines and organophates) resulted in bed bugs becoming a minor pest restricted to primitive localities and places with unsanitary conditions (Ebeling 1978, Moore and Miller 2006). By the 1970s the once ubiquitous bed bug, found in all 50 US states, had fallen from its position as a major pest of hotels and homes (Snetsinger 1997). However, the occurrence and prevalence of bed bugs would dramatically change later that same century.

During the late 1990s the first reports of resurgence in bed bug infestations started to appear. At the international level, places as far away as England and Australia reported growing bed bug problems (Reinhardt and Siva-Jothy 2007, Doggett and Russell 2008, Richard et al. 2009). Similarly for North America, the same increase in bed bug infestations was also reported (Cooper and Harlan 2004, Boase 2001, Gulmahamed 2002, Cooper 2011). Subsequently nationwide pest control industry surveys also reported on the phenomenon of a bed bug "plague" (Potter et al. 2010, 2011). Media attention escalated on the bed bug resurgence in dorms, hotels, and public buildings in North America; some reports even mentioned the closing of department stores (Roberts and Burke 2010). Today, we all have read or heard the accounts of terrified homeowners or renters about receiving bed bug bites and the misery they have endured from infestations. In response to bed bug outbreak throughout North America, the US Environmental Protection Agency and US Centers for Disease Control and Prevention issued a joint statement on the growing bed bug problem and need for control (http://www.cdc.gov/nceh/ehs/publications/bed_bugs_cdc-epa_statement.htm). Interestingly, the exact causes for the renewed prominence of bed bugs are not known; however, some of the important factors

include increased international travel, pesticide resistance, ease in which they are spread, and a reduction in the indoor use of residual insecticides (Cooper 2011, Jones and Bryant 2012).

In California, resurgence in bed bugs has been recognized and reported on by State and county public health officials (Ojo 2008, Anonymous 2011, Anonymous 2012a), pest control industry trade magazines (Getty 2007, 2010; Anonymous 2010; Hooper 2010; SPCB 2010a; Lewis et al. 2012), and the University of California (Lewis et al. 2009). Media outlets in the State have also featured articles and news segments on the bed bug resurgence (Anonymous 2009, 2012b; de Brito 2010). Although State authorities, pest control industry, and University of California all agree that a significant increase in bed bug infestations has occurred, detailed surveys on the prevalence and intensity of infestations in hotels, apartments, college housing, public buildings, and transportation facilities are lacking.

Because of the resurgence, public health, and annoyances associated with bed bug infestations and bites, there has been a refocusing on understanding their biology and ecology. Much of what is known on the basic biology and ecology of bed bugs was conducted prior to 1966 (Usinger 1966). Over the last decade, modern technology and computer-aided data acquisition and processing have made laboratory research easier. However, much of the recent work on bed bug chemical ecology (Siljander et al. 2007, 2008; Olson et al. 2009; Weeks et al. 2010a), electrophysiology (Levenson et al. 1974, Harraca et al. 2010a), host-seeking behavior (Suchy and Lewis 2010), characterization of infestations and dispersal (Pfiester et al. 2009; Wang et al. 2009a,b, and 2010; How and Lee 2010), just reaffirm previously reported work going back four decades. Published research studies that are unique to this century include the use of molecular makers to demonstrate the origin of bed bugs in North America (Booth et al. 2012), population genetic structure (Saenz et al. 2012), documenting resistance to pyrethroid insecticides (Romero et al. 2009, Zhu et al. 2010), nymphal cuticular and anti-aphrodisiac extracts (Dominque et al. 2010, Harraca et al. 2010b), and efficacy of canine (Pfiester et al. 2008) and portable monitors (Anderson et al. 2009, Wang et al. 2010, Weeks et al. 2010b, Wang and Cooper 2011, 2012). Several papers included tests of pesticide products and localized heat (Moore and Miller 2006, Benoit et

al. 2009, Mix 2010, Pereira et al. 2009); however, since the 1970s only four new active ingredients (chlorfenapyr, dinotefuran, hydroprene, and imidacloprid) have been registered and widely used for bed bug control (Potter et al. 2011, Cooper 2011).

Regardless of the origins of the current bed bug resurgence, remedial control and preventative actions that involve many buildings and contents over a wide area will require the accurate detection and monitoring of infestations. Visual searches assisted with flashlights traditionally have been the primary method for confirmation of, and monitoring for, bed bugs (Potter 2006, Pinto et al. 2007, Cooper 2011). The visual evidence used to a verify bed bug infestations includes the actual sighting of adult and immature stages, as well as eggs (Cooper 2011). The detection of bloodstains or bed bug fecal material is also diagnostic for the presence of an infestation. The presence of bites is used to a lesser extent because humans have wide and varied responses to bed bug bites, from no reaction to anaphylactic shock (Klotz et al. 2009, Cooper 2011). Recent industry surveys document and support a reliance on visual searches, as much as 99% in 2011 (Potter et al. 2011). However, in those same surveys, a growing trend in adopting other methods of detection and monitoring were noted that include active traps, passive traps, and canine scent detection.

Over the last several years there has been heighten interest in identifying and characterizing the monitoring process for bed bugs, including the use of humans, canines, and devices. Although human visual searches dominate bed bug detection and monitoring methods, upon critical evaluation they have proven to be very labor intensive and seriously underestimate bed bug numbers, especially at low infestation levels (Pinto et al. 2007; Wang et al. 2009a, 2010; Weeks et al. 2010b; Cooper 2011; Lewis unpublished data). For human searches, the degree of clutter, cleanliness, and scale of area searched are limiting factors when searching for organisms that are less than 2 mm in size (eggs and small immatures). Studies that evaluated canine scent detection have been conflicting; laboratory investigations suggest a high degree of correct bed bug detection, 99% (Pfiester et al. 2008), but averaged 43% for actual field studies (Wang and Cooper 2011). For canines, the actual chemical stimuli detected are still being

debated, but probably involves at least the bed bug alarm pheromones, (*E*)-2-hexenal and (*E*)-2-octenal (Feldlaufer et al. 2010). There have been few papers that critically evaluated the performance of passive (containing no lures or attractants) and active (containing bed bug lures and attractants) monitors (Wang et al. 2010, Wang and Cooper 2011, 2012). These studies presented findings on three active and one passive monitor. Their findings were mixed and were heavily dependent of preexisting bed bug infestation levels and monitor placement. Because of the resurgence in bed bugs and people becoming more apprehensive or not willing to accept close contact with pesticides (Pereira et al. 2009, Potter 2011), efficacious monitors are essential (Weeks et al. 2010b).

In direct response to the bed bug resurgence in California, and following an industry plea, the Structural Pest Control Board (SPCB) authorized a request for research proposals to address this specific problem (SPCB 2010a,b). The study that follows was awarded research funding to conduct investigations into the performance of bed bug monitors. The investigations included laboratory and simulated-field testing. Both types of monitors, passive and active, were included in the tests. The large-scale testing capacity for the simulated-field investigations using densities of 10, 50, and 100 bed bugs made this study unique. Then introducing adult bed bugs into the testing arenas with several furniture items added realism, 3-dimensional strata, and robustness to our findings. Our hypotheses, including monitor performance, were functions of differences in bed bug feeding condition and foraging behavior between sexes. Our finding will aid to clarify and characterize the reliability and robustness among monitor types, especially when used as tools for early detection of low-density bed bug infestations, and pre- and post-treatment monitoring and evaluation.

MATERIALS AND METHODS

Bed bug source

Live adult male and female bed bugs were purchased from Sierra Research Laboratories in Modesto, CA. This laboratory has a federal permit to rear bed bugs on both artificial rearing medium and

laboratory kept mammals. The particular strain we used for testing was called "Earl", and was field collected from Modesto, CA in 2007. This field strain has been in colony for five years and, based on Sierra Research Laboratory tests, is susceptible to pyrethroids, imidacloprid, and propoxur (Sierra Research Laboratory, unpublished data). The sex of bed bugs was determined by staff at the Sierra Research Laboratory and designated on the shipment container to our lab. To help minimize research costs ($2.50/bed bug) any surviving bed bugs were returned to the lab colony to be re-cycled for use in future testing so long as they were healthy and feeding.

Bed bug handling and feeding method

Polystyrene jars (5.2-cm dia; 59 ml; Consolidated Plastics Company, Inc., Stow, OH) were modified for containing bed bugs. A hole was drilled in both the base of the jar and in the screw top lid, which were covered using fine-nylon-mesh screen (Plankton Netting rated at 73 squares per inch; BioQuip, Gardena, CA), and that was secured by hot glue and then reinforced with electrical tape. Several pieces of cardboard were provisioned to each jar as harborage sites.

Bed bugs were fed, as necessary, using an artificial feeding method adapted from Montes et al. (2002) and Chin-Heady (2010), using heated blood through a Parafilm® membrane (Fig. 1). Food-quality pork blood came packaged in 1-gallon (3.78 *l*) containers (Yosemite Meat Company, Inc., Modesto, CA) and preserved with sodium citrate to prevent blood clotting. Blood for this study was stored in an upright two-door refrigerator-freezer (GE No-frost refrigerator-freezer, Fairfield, CT; capacity 0.52 m^3) at 3 ^oC.

For feeding bed bugs, blood was poured into a clear plastic box lid (Tri-State Plastics Inc., Dixon, KY) and covered with a stretched piece of Parafilm"M"® (Neenah, WI) and was heated on an electronic hot plate (Isotemp, Fisher Scientific, Waltham, MA). When the blood reached a temperature between 37-40^oC, jars containing bed bugs were placed on top. Each feeding session lasted for up to 20 minutes;

however, if bed bugs had not fed or their abdomens were not visually distended, they were presented with a newly prepared blood meal.

After feeding, bed bug jars were kept in an incubator (Fisher Scientific, Waltham, WA) set at 30^{o}C, an optimal temperature for maintaining bed bugs (Usinger1966). All handling and transfers of live bed bugs were conducted within a stainless steel pan, using paintbrushes (Liquitex Artist Materials, Piscataway, NJ), and featherweight, soft-tipped forceps (BioQuip Products, Rancho Dominguez, CA). An anti-crawling liquid material (Insect-A-slip, BioQuip Products, Rancho Dominguez, CA) was applied to the top 2.4 cm of the pan as an additional safety measure to prevent bed bug escape.

Monitors

The monitors used in the screening trials included the following devices (nickname in parentheses): BB Alert® Passive (BBAlert) (MidMos Solutions Ltd., Brierly Hill, West Midlands, UK), Bedbug Detection System (BDS) (Catchmaster™, AP&G Co., Inc., Brooklyn, NY), Climbup® Insect Interceptor (Climbup) (Susan McKnight, Inc., Memphis, TN), BB Catch™ (BBCatch) (BioTrap™ Science, Portland, Oregon), and NightWatch™ (Nightwatch) (BioSensory, Inc., Putnam, CT) (Fig. 2). The first three monitors mentioned BBAlert, BDS, and Climbup are classified as passive monitors, meaning they do not contain any attractants or lures (Weeks et al. 2011b).

The BBAlert is made of a piece of cardboard (9.5 by 6.6 by 0.3 cm) that is housed in a two-piece plastic frame that has a slightly larger bottom with an adhesive back (Fig. 3A). The cardboard provides a refuge location for bed bugs to wander in and out of. The BDS is made from a piece of cardboard (15.5 by 4.4 cm) (Fig. 3B) with a 1.8-cm wide adhesive strip on the back to attach the monitor onto walls or furniture. Each cardboard strip is folded in half before placement, and creates a thin cardboard sandwich (7.5 by 4.4-cm) containing ten 1.25-cm diameter adhesive dots to entrap wandering bed bugs. Climbups used during the laboratory portion are white in color and made of two concentric dishes (11.3 dia. and 15.2 dia.) with a 2.3-cm band of roughened white tape applied around the outer exterior wall as a surface

conducive for bed bugs to crawl on (Fig. 3C). The walls of the outer and inner dishes create a pitfall trap designed to capture bed bugs foraging from surrounding room locations. Furniture legs can be placed inside of the inner dish that is designed to capture and isolate bed bugs originating from that piece of furniture.

The two active monitors tested contain lures and attractants (Weeks et al. 2011b), each one employing the use of heat, CO_2, and chemical lures and required access to a standard electrical outlet to operate. The BBCatch is a plastic briefcase-shaped box (29.5 by 26.7 by 7 cm) (Figs. 2, 4A). Inside the unit is an L-shaped metal-housed control panel where there is access to timer controls, an opening for a small canister of compressed CO_2, designed by the manufacturer to last for 8 hours, and a capture slide (5 by 5 cm) lined with adhesive mixed with chemical lure. The capture slide is placed onto a heated metal support that is attached to a piece of metal wrapped in black cloth and extends to the floor through a small opening (1 by 2 cm) in the back of the unit's carrying case. Above the capture slide is a hose where CO_2 is released (Fig. 4A).

The NightWatch™ monitor was the largest monitor tested (35 by 24 by 24 cm) and uses a blend of heat, chemical attractants, and CO_2 from a standard sized cylinder (available at any paintball or sporting goods supply store) (Fig. 4B). The monitor has one main hollow housing unit with an LED display control panel at the front, an open back where the CO_2 cylinder is threaded, and has two flanking detachable pit fall traps. The body of the unit is heated, including the sidewalls and the top where the lure is placed and from which CO_2 is emitted. Each pitfall trap has a 45^o inclined ramp is covered by a 22- by 5.4-cm strip of felt (Fig. 4B).

We chose these five monitors for our laboratory study because most have some published findings of their utility, and they also represented a good balance between passive and active monitors that are sold and used by pest management professionals (PMPs) in California. For additional details on these products and specifications see the following papers and websites (Wang and Cooper 2011, 2012;

Weeks et al. 2010b; www.bedbugsalert.com; www.catchmasterpro.com/bds.php; www.insect-interceptor.com, www.biotrapscience.com; and www.biosensory.com).

Laboratory investigations

Laboratory-testing arenas

Three large containment chambers (2 x 0.3 x 0.3 m; Fig. 5) were built to test monitor performance and observe bed bug foraging movement. Arena floors were made from a single, construction-grade Douglas-fir board (2.4 m by 0.3 m by 0.05 m; Home Depot, El Cerrito, CA) painted with a clear varnish (Satin Brushing Lacquer, Deft, Irvine, CA), and clear polycarbonate plastic walls were attached (Tap Plastics, Inc., El Cerrito, CA). The two long walls (2.2 by 0.3 by 0.005 m) were secured to the wood base along the length of each board using seven equidistant sheet metal screws (3mm by 16-mm long). The two long walls were then screwed into the two shorter perpendicular walls (0.3 by 0.3 by 0.01 m) that rested on top of the wood base with three evenly spaced sheet metal screws (3 mm by 18 mm long). Any wide gaps between surfaces were filled with weather stripping (6.3 mm by 12.7 mm; Foam Tape, M-D Building Product, Inc., Oklahoma, OK) and all edges were sealed with white adhesive caulk (Kitchen and Bath caulk, Dap, Baltimore, MD). The top 7.6 cm of the walls were treated with anti-crawling liquid material (Insect-A-slip, BioQuip Products, Rancho Dominguez, CA) and the entire containment chamber was covered with screen netting (Fig. 5).

Laboratory testing methods

These trials were conducted in a small, detached, one-room building known as the insectary. The rectangular building has windows on the longer northeast and southwest facing walls and one door each on the shorter adjacent walls. The room is not insulated, was not temperature controlled, except for a small space heater near the main work area of the lab technician, who maintained normal working activities. The testing arenas were oriented lengthwise northwest to southeast and were placed 3.3 m

from the work desk; monitors were placed at that end of the arena. The bedbugs were placed at the opposing side, further away from the workspace.

In total, we evaluated three factors: monitors (five total plus untreated checks), sex (male or female), and feeding condition (fed or staved). Only male bed bugs were marked with a water-based, nontoxic correction fluid (Wite-Out®, MMIX BIC USA, Inc., Shelton, CT) to facilitate sex identification during data collection. Fed condition was defined as being fed on pork blood three days before testing, and starved was defined as having gone one to two weeks without a blood meal.

All three replicates of each monitor and the untreated check were conducted at the same time; however, monitors were randomly selected at the beginning of each 3-replicate trial. Because they contained no attractants or lures, the three replicates of the passive monitors and untreated checks were evaluated simultaneously, three at a time. In contrast, active monitor replicates were conducted one at a time so that the monitor in one individual test chamber did not affect the responses in an adjacent chamber. There were 36 total replicates for all monitor and feeding condition combinations: three replicates each of six monitors (five monitor types and an untreated check) by two feeding condition combinations. Between trials, chambers were vacuumed and all surfaces of the testing chamber were wiped down with 70% isopropyl alcohol (Swan, Smyma, TN) to remove any lingering odors.

For each replicate, ten bed bugs (five males and five females) were provisioned with a small piece of unbleached cardstock (2.54 x 3.81 cm) from the colony jar that served as a harborage site and placed in arenas in the afternoon. Contained underneath a screen-covered screw top lid (5.2-cm dia.; Consolidated Plastics Company, Inc., Stow, OH) the bed bugs acclimated overnight. The bed bugs were released the following morning and were free to move and forage within the testing arena until the following afternoon. During the observation period, five visual observations were conducted; for each one we counted the number of bed bugs (by sex) inside or on the harborage, inside or on the monitor, and on the floor of test chambers. All observations were made during regular work hours (0830 to 1730 hrs).

Additionally, locations of bed bugs found on the floor were given distance value, measured in cm from the southwest corner of each arena.

Variables of interest and statistical analyses

A repeated measures generalize linear model (ANOVA, SAS Institute 1994) was used to compare and analyze all treatment effects. An F-statistic (SAS Institute 1994) was used to accept or reject the null hypotheses that no differences existed among bed bug counts and treatment effects that included monitor (including untreated checks), sex, and feeding condition. Frequency distribution plots of bed bug counts and means for all monitors and untreated checks were conducted using computerized software (R Development Core Team. 2004). Multiple comparisons for mean percentage counts among monitors by gender and feeding condition were conducted with Tukey's test (SAS 1994).

Simulated- field investigations

Villa Termiti preparations

The Villa Termiti (Building 475, UC Richmond Field Station, Richmond, CA) was built in 1993 from funding provided by the California Structural Pest Control Board to evaluate the efficacy of nonchemical control methods for drywood termites. The building is 37.2 m^2 in size; has four symmetrical walled sides in the main room, each containing two windows and one door; attic; and subarea (Lewis and Haverty 1996) (Fig. 6A). To accommodate the release of live bed bugs into the Villa Termiti, several modifications were made and included: installation of new drywall and floor in the main room to contain bed bugs. Bed bugs are known to withdraw from light (Usinger 1966) so we covered two doors and all eight windows with drywall so we could control lighting and entry points into the Villa Termiti. After repairs to the plywood subfloor, linoleum was installed (Fig. 6B). The nightshade blue color of the linoleum was chosen to facilitate locating adult or immature bed bugs. To minimize seams, only three large pieces (2 m by 6.1 m) of linoleum were used. An additional containment feature included a 15-cm

rubber base along the walls. During all testing, only authorized personnel were allowed entry; at all times the testing structure was locked and a sign posted restricting entry (Fig. 6A).

Constructing testing arenas

The actual release of live bed bugs was restricted to four large wooden arenas lying on the Villa Termiti floor (Fig. 7A). Each test arena was built using two 4-ply, Douglas-fir plywood sheets (2.4 m by 1.1 cm by 1.2 m (lwh)) (Home Depot, El Cerrito, CA). Before assembly, each plywood sheet was lightly sanded; large knots and crevices filled with wood filler (Carpenter's Wood Filler, Elmer's, Columbus, OH), and finally at least three coats of a quick drying clear wood finish (Satin Brushing Lacquer, Deft, Irvine, CA) were applied. To secure the two sheets of plywood together, they were laid on the floor along their long sides, and the center seam was filled with white adhesive caulk (Kitchen and Bath caulk, Dap, Baltimore, MD) and reinforced with a long piece of adhesive tape (Duct Tape, Henkel Consumer Adhesive, Inc. Avon, OH) after the caulk fully dried. To further stabilize the center seam area, the two adjoining pieces of plywood sheets, at both the north and south ends, were fastened together using a single 15- by 1.8-cm zinc coated metal bar (Stanley Supply & Services, Inc., North Andover, MA) using four 4-mm diameter by 1.5-cm long slot-headed wood screws (Home Depot, El Cerrito, CA; Fig. 7B).

The four vertical walls of the testing arenas were made from clear 2.36 m by 0.48 cm by 10.2 cm (lwh) polycarbonate plastic strips (Tap Plastics, Inc., El Cerrito, CA) (Fig. 7A). Each strip was affixed to the plywood sheets using ten 11-mm wide zinc coated metal L-braces (Everbilt, City and State) that were evenly spaced 30.4 cm longitudinally and to an inside depth of 3.8 cm along the entire edge (Fig. 7B). Each brace was held in place to the plywood sheet using two 1.27-cm long; 3-mm diameter zinc coated slotted wood screws (Crown Bolt, Aliso Viejo, CA). In total, 40 metal L-braces were needed to secure all four vertical plastic sides to the wooden floors for each test arena. The opposite ends of each L-brace were secured to the plastic strip using a single 4.7- by 15.8-mm, slotted round machine screw (Home Depot, El Cerrito, CA) and 4.7-mm diameter wing nut (Home Depot, El Cerrito, CA). For additional

stability, each corner of the testing arena was held together with a single 11-mm metal L-brace that was drilled and fitted with two 4.7-mm diameter slotted round machine screws (Home Depot, El Cerrito, CA) and wing nuts (Home Depot, El Cerrito, CA) (Fig. 7b). All exterior and interior edges where the plastic walls meet the wooden floor and corner seams where the two plastic walls touched were sealed with white adhesive caulk (Kitchen and Bath caulk, Dap, Baltimore, MD). Larger gaps underneath plastic walls were sealed with foam weather stripping tape (6.3-mm by 12.7-mm; M-D Building Product, Inc., Oklahoma, OK). Additional bed bug containment measures included the installation of a 2.5-cm wide overhang made from adhesive tape (Duct Tape, Henkel Consumer Adhesive, Inc. Avon, OH) to the top edge of all walls (Fig. 7B).

Test protocol and design inside wooden arenas

A wagon-wheel design was used that featured a central release point for bed bugs from which they could forage outwards from the harborage towards any items placed in testing arenas (Fig. 7A). We adopted this design from Rust and Reierson (1977), who used a similar design to test the repellency of pesticides to cockroaches. The advantages of using this design included a central release point for insects that allowed them equal distance to the first point of contact to items placed in the test arenas. For our simulated-field tests, the release point was centrally located at the centers of the 2.4- by 2.4-m wooden plywood sheets.

To add realism to testing, a wooden bed (85 cm by 75 cm by 22 cm (lwh); Kriter; IKEA, Emeryville, CA), table (63 cm by 48 cm by 45 cm (lwh); Latt, IKEA, Emeryville, CA), and small cotton fabric towel that served as a rug (fast dry towel, 71 cm by 66.6 cm; RE Style Room Essentials, distributed by Target Corporation, Minneapolis, MN) were added inside the testing arenas. The bed was solid pine, had a clear acrylic lacquer finish, mattress (85 cm by 70 cm by 9 cm (lwh); Vyssa Slummer, IKEA, Emeryville, CA) and bed bug mattress protector (London Luxury Bed Bug Mattress Protector Encasement, New York, NY). The table was also made of solid wood but did not have a finish. The rug

was made of 100 % cotton and was machine washable. The original length of the bed, mattress and rug were longer but were cut to the sizes reported in this study in order to fit the inside dimensions of the test arenas. Additionally, the ends of the rug were sewn with a sewing machine to seal any frayed ends that could conceal bed bugs from view and act inadvertently as harborage locations during tests.

All furniture item and monitor placement positions were randomly assigned to a quadrant and their corners placed at a 38.1-cm distance from the central release point. To minimize the effect of furniture orientation, their four corners were also randomized before placement in the test arenas. For tests involving Climbup and BDS products, a second monitor was installed on the leg of the bed closest to the arena wall and in the opposite quadrant of the first monitor (Fig. 7C). The manufacturer's use instructions for these products recommend the placement of their products on the legs of furniture items for maximum monitor performance.

Releasing bed bugs and data collection

The release and treatments in the simulated-field tests included varying the density of bed bugs and monitor products. The bed bug strain (Earl) used for testing came from the same supplier as for the laboratory phase, Sierra Research Laboratory (Modesto, CA). Only starved adult males and females were used. The adult bed bugs were one to two weeks starved before being released in the test arenas. The density of bed bugs used was 10, 50, and 100 per arena. The sex ratio in test arenas was 1:1, male to female. Three monitors were used during tests and included Bedbug Detection System (BDS)(Catchmaster™, AP&G Co., Inc., Brooklyn, NY), Climbup® (Climbup) HD Insect Interceptor (Hotel Discreet; Susan McKnight, Inc., Memphis, TN), and NightWatch™ (Nightwatch) (BioSensory, Inc., Putnam, CT). We used these three monitors based on their performance in our laboratory trials, and in the case of the single active monitor, it was the only active monitor registered for use and sale in California during our study. There were two modifications to the climbups used during laboratory-testing phase and the simulated field tests. Climbup HDs used in the Villa tests are smaller with a 9.8-cm

diameter and an inner diameter of 7.5 cm. They are dark black in color and a black, soft band of Velcro was wrapped around the exterior wall (Fig. 3D). All monitor product assignments and positions in the test arenas were randomized.

In total there were two treatments, type of monitor and density of bed bugs. All treatments were replicated 3 times for the low and medium bed bug densities for passive monitors and untreated checks, and due to the limited time to complete the study, only two times for all active monitor densities and for the highest density for passive and untreated checks. Live, starved adult male and female (ratio 1:1) bed bugs in densities 10, 50 or 100 were released in the center of testing arenas. A small piece of cardboard (7.6 cm by 10.2 cm) was also provided so the bed bugs had a harborage while in the test arenas. After acclimating overnight, the bed bugs were released and allowed to move about the test arenas and contents for 24 hours.

For passive monitors, up to three testing arenas were simultaneously used to collect data. For active monitors, and because of their confounding effect over a large area, only one arena was used and the entire Villa Termiti space was considered a single replicate. To aid the visual sighting and counting of bed bugs in test arenas, adult male and female bed bugs were marked with two different colors of water-based, nontoxic paint pen (Sharpie, Oak Brook, Il). To further aid the counting process and for documenting bed bug locations within test arenas, the arena was divided into sixteen square grids of equal size (0.6 m by 0.6 m) that were also given a unique location designation (1-16).

During the 24-hour observation period, three visual observations were conducted. At each observation the location of any seen bed bugs was documented. At the final visual observation, the monitors and cardboard harborages were collected, all furniture items were searched and disassembled and searched again, and the remaining floor space was visually checked. Counts of bed bugs were made for each furniture item, harborage, monitors and floor space, and recorded based on their floor grid location. Between replications, all surfaces within the testing chambers and wooden furniture were

vacuumed (Shop-Vac, Williamsport, PA and/or ProTeam backpack vacuum, Boise, ID) for any remaining bed bugs or eggs, wiped down with 70% isopropyl alcohol (Swan, Smyma, TN), and also wiped down with a bleach solution (0.05%) (Clorox, Oakland, CA), to remove any lingering bed bug odors. Lastly, any minor repairs to seams or attachments between plastic walls and wooden floor were made as necessary. The rug, cleaning rags, and any other durable utensils or equipment used were either heat sterilized using a portable heater (48 cm by 91 cm by 61 cm (lwh), Packtite™ Portable Bed Bug Killing Heater, Nuvenco, Inc., Fort Collins, CO), put inside a freezer overnight, or washed with soap and water.

Variables of interest and statistical analyses

A repeated measures generalize linear models (a type of ANOVA, SAS Institute 1994) was used to compare and analyze all treatment effects. An F-statistic (SAS Institute 1994) was used to accept or reject the null hypotheses that no difference exists among monitors and varying densities of bed bug counts when compared to untreated checks. Plots of the percentage of bed bugs found among monitors and furniture items, gender percentages found among monitors and furniture items, percentage bed bugs found among monitors and density released, and percentages of bed bugs found among interior floor cells were conducted using computerized software (R Development Core Team. 2004). Comparisons for mean percentage counts among monitors by furniture items were conducted with individual t-tests (SAS 1994).

RESULTS AND DISCUSSION

Laboratory investigations

The mean percentage of bed bug counts found among the five monitors tested ranged from 27.7 to 33.7% and were not significantly different ($F = 2.04$; $DF = 5/50$; $P > 0.08$) from the mean percentage for the untreated check (30.9%)(Table 1). There were also no significant differences between sexes or feeding conditions ($F = 0$; $DF = 1$; $P > 0.99$ and $F = 0$; $DF = 1$; $P > 0.99$, respectively) among monitors compared to untreated checks (Table 2). Admittedly, the results from the laboratory phase of our investigations are unremarkable; however, we were pleased to observe bed bug foraging in the test arenas

(on average 66% of time away from harborage location) for a wide range of ambient temperatures (11 to 28^{o}C), monthly variance in observations (November 2011 to February 2012), and also during day light hours. Prior studies report difficultly in getting bed bugs to forage in laboratory settings and show that bed bugs spend up to 90% of their time in harborage locations hidden from view (Cooper 2011). Our experiences were opposite; soon after release, bed bugs could be seen actively moving about exploring the interiors of the test arenas, monitors, and harborages provided. Although not significant a trend in increasing bed bug counts was observed for the BDS monitors, even more so when compared to the two active monitors, BBCatch and NightWatch (Fig. 8). Our explanations for this anecdotal observation involves the primarily cardboard composition of the BDS monitors and the thigmotactic response of bed bugs to tight harborage spaces (Usinger 1966), and scarcity of harborage locations in the test arenas. What we were able to glean from the laboratory results was that the "Earl" strain had robust and active foraging behavior over a wide range of temperature and ambient lighting conditions regardless of sex or feeding condition. Based on these results we went forward with the simulated-field conditions in the Villa Termiti using two passive monitors, Climbups and BDS, and one active monitor, NightWatch. Our reasons for going forwarded with the three monitors included the availability and use of Climbups and BDS in California by pest control operators, and the opportunity to compare our laboratory findings to simulated-field testing in the Villa Termiti.

Simulated-field investigation

Results from our simulated-field tests were similar to laboratory findings and can be characterized as none of the monitors captured as many or more bed bugs than the original harborage release source or ground/floor of test arenas (Fig. 9). The mean percentage of bed bugs captured was 7% for BDS, 11% for Climb-up, and 12% for NightWatch™. These differences were not statistically significant ($F = 0.21$; $DF = 2/13$; $P > 0.81$). Looking at Figure 9, the line trace for Nightwatch was the lowest among monitors tested. This device is categorized as an active monitor and contains several attractants including heat, chemical lure, and CO_2 (Weeks et al. 2010b). Our results with NightWatch™

and its poor attraction to bed bugs corroborates a previous published finding (Wang and Cooper 2011). The reasons for the poor attractiveness and performance for NightWatch™ in our simulated-field tests may involve the volume and scale of the Villa Termiti and test arenas (volume 154 m^3, Lewis and Haverty 1996) and the difficulty in achieving the needed concentration of CO_2 and other airborne chemical lures to draw bed bugs into the monitor. One last complication that affected the performance of the NightWatch™ was the ease with which bed bugs conceal themselves within the device. A week after conducting our last experiment with the NightWatch™ at a density of 50 bed bugs, when the equipment was being moved for storage, we found two marked females hidden inside the device. Earlier, these same insects were recorded as missing data and lost to further analyses. The two insects, when discovered, were destroyed, but not until after one of the authors discovered 9 bites at home. Fortunately, of the 1,460 bed bugs released and tested during the study, only 11 were unaccounted for (recapture rate 99%). However, our unaccounted bed bugs results reinforce other studies that attest to the tremendous adaptability of this public health pest and its ease of spread far removed for the originating source. Future CO_2 emitting devices will need to be built of materials and compartments that resist hiding and concealment of bed bugs.

Over the last several years, our experiences with attractive monitors containing CO_2 have been mixed or conflicting. There are several published papers that report increased capture of bed bugs when using CO_2 in the form of dry ice (Wang et al. 2009, Wang and Cooper 2011) or as compressed gas (Anderson et al. 2009). There are other papers that report no or poor attraction ability when using CO_2 in the form of dry ice (Lewis, unpublished data) or compressed gas (Wang and Cooper 2011). Our lab has also published a paper on the use of CO_2 generated from a human source that was highly attractive (Suchy and Lewis 2011). Obviously additional research is needed to clarify the role of CO_2 and generating source when used as an attractant in monitors for bed bugs.

A second monitor performance finding from our investigations involved the color of the Climbup® Insect Interceptors. We used white colored Climbups for our laboratory studies and black

Climbups for our simulated-studies because we were informed by the manufacturer that the black version would increase capture performance based on a previous study (Wang and Cooper 2012). Looking at our capture results for the white versus black Climbups versions 28% and 20% respectively, we did not find a relationship between increased performance and monitor color (Table 1 and Fig. 9).

Varying the density of bed bugs did not result in statistically significant differences in the percentage capture (Fig. 10). The relationship among varying densities of bed bugs and mean percentage captured was not statistically significant ($F = 0.85$; $DF = 2/13$; $P > 0.45$); although a downward trend was observed for the two passive monitors at the higher densities. Viewing the y-axis values for mean percentages found, the capture rate for bed bugs among monitors hovered between 3.4 to 25.6% over the three densities tested. Our inference drawn from this finding is that attempts to extrapolate bed bugs captured in monitors to actual field conditions and natural populations levels in rooms or structures is an underestimation. Based on our capture data for monitors and known population levels, under actual field conditions, 4 to 20-times more adult bed bugs could be hiding or concealed from view. Combining our results with the finding that nymphs represent at least 78% field populations (Wang et al. 2010), could justify a 30-times or higher inflation factor to use when estimating field populations based on contained in monitors.

Bed bug foraging behavior

Analyses of individual insect positions tested inside arenas clearly revealed bed bugs actively foraging among all furniture items and especially among the interior locations on the floor (Figs. 9 and 11). At less than 5%, the lowest percentage of bed bugs was found in or near the bed and highest 15% for the table (Fig. 9). However, among arena contents, the highest percentage of bed bugs was found at the harborage source. This is an obvious finding given that the bed bugs were initially released into the arenas from the centrally located harborage source. The distribution of bed bugs on the floor was mostly concentrated within the four interior cells spaces nearest the harborage source and release site (Fig. 11).

There were no statistically significant differences in dispersion of bed bug adults on the arena floor in relation to the different monitors ($F = 0.67$; $DF = 2/18$; $P > 0.52$).

Based on our laboratory and simulated-field findings, bed bugs had moved at least 60% of their time away from their harborage location, irrespective of ambient temperature, month of year, and feeding condition. These findings were expected given bed bug random foraging behavior (Usinger 1966), detection range of hosts within 150 cm (Usinger 1966), and all furniture items being positioned 38.1 cm from the harborage source (Fig. 7A). Interestingly, there was a statistically significant difference between the percentage of the sexes among monitors used for all furniture items, floor/ground, and also for all furniture items in the test arenas for the controls (Table 3). These findings support the conclusions that males spent most of their time in or near the harborage source (Fig. 12). Therefore, it is the females that spend more time wandering farther in rooms, apartments, and structures than males; this behavior involves the need of the females to find oviposition sites (Usinger 1966, Suchy and Lewis 2011) and to escape the debilitating and sometimes lethal mating attempts, i.e. traumatic insemination, by males (Usinger 1966, Cooper 2011).

Combing lab and simulated field results

So what have we learned about bed bug foraging behavior and monitors based on our research? Compared to other methods and studies published on bed bug foraging, our bioassay methods used were unique in that they included test building scale, pest density, 3-D setup of furniture items, and wagon-wheel choices presented to bed bugs. Our simulated-field methods and findings also demonstrated that visual searching for bed bugs is very laborious and some will be missed in inspections. Even with considerable redundancy built into the Villa Termiti to contain foraging bed bugs, some escaped, were missing, or were transported to far away locations. We are comfortable that our findings and conclusions were robust and are applicable to what PMPs can expect to see from bed bugs foraging under actual field conditions. We also showed that among the five monitors tested, none was found to demonstrate

attractive ability over distance for the three densities of bed bugs tested. Basically, our tests showed that monitors were nothing more than a new piece of furniture that bed bugs randomly explored during foraging forays. At best, only statements on the presence or absence of an infestation can be made; statements concerning low-density infestations and bed bug population size are speculative. There is still a need in the marketplace for a proven attractive monitor, especially to evaluate the presence and existence of low-density infestations. Perhaps future products, when they are field tested and available to PMPs, will fill this need. In the interim, when monitors are used for bed bugs, more per room or space is better than fewer, and deployment for longer monitoring times is best.

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TABLES

Table 1. Mean percentage counts (± STD error) for the bed bug, *Cimex lectularius*, among five monitors and untreated checks tested during laboratory investigations.

Monitor[1]	% Bed Bugs[2]
BB Alert	33.7 ± 3.3%a
BB Catch	27.7 ± 2.7%a
BDS	33.2 ± 3.2%a
Climbup	28.5 ± 2.7%a
NightWatch	29.4 ± 2.9%a
Untreated checks	30.9 ± 3.2%a

[1]BB Alert = BB Alert® Passive (MidMos Solutions Ltd., Brierly Hill, West Midlands, UK), BB Catch = BB Catch™ (BioTrap™ Science, Portland, Oregon), BDS = Bedbug Detection System (BDS) (Catchmaster™, AP&G Co., Inc., Brooklyn, NY), Climbup = Climbup® Insect Interceptor (Susan McKnight, Inc., Memphis, TN), and NightWatch = NightWatch™ (BioSensory, Inc., Putnam, CT). Laboratory tests were conducted in Richmond, CA.

[2]Means followed by the same letter were not significantly different at the 5% level using Tukey's test (SAS 1994).

Table 2. Mean percentage counts (± STD error) by gender and feeding condition for the bed bug, *Cimex lectularius*, among monitors and untreated checks tested during laboratory investigations in Richmond, CA.[1,2]

Monitor[1]	Fed Bed Bugs[2]		Starved Bed Bugs[2]	
	Male	Female	Male	Female
BB Alert	27.6 ± 5.1%a[3]	31.8 ± 5.7%a	37.5 ± 7.7%a	38.5 ± 7.8%a
BB Catch	28.9 ± 5.5%a	23.5 ± 4.6%a	33.3 ± 6.4%a	25.3 ± 5.2%a
BDS	39.1 ± 7.2%a	35.9 ± 6.6%a	33.3 ± 6.4%a	25.4 ± 5.2%a
Climbup	31.8 ± 5.7%a	29.2 ± 5.4%a	28.1 ± 5.6%a	25.0 ± 5.1%a
NightWatch	32.3 ± 5.9%a	29.4 ± 5.5%a	28.9 ± 6.3%a	27.3 ± 6.0%a
Untreated	35.3 ± 6.7%a	26.5 ± 5.4%a	33.3 ± 7.0%a	28.9 ± 6.3%a

[1]BB Alert = BB Alert® Passive (MidMos Solutions Ltd., Brierly Hill, West Midlands, UK), BB Catch = BB Catch™ (BioTrap™ Science, Portland, Oregon), BDS = Bedbug Detection System (BDS) (Catchmaster™, AP&G Co., Inc., Brooklyn, NY), Climbup = Climbup® Insect Interceptor (Susan McKnight, Inc., Memphis, TN), and NightWatch = NightWatch™ (BioSensory, Inc., Putnam, CT). Laboratory tests conducted in Richmond, CA.

[2]Feeding condition defined as fed having a blood meal 2-3 days and starved going 7-14 days without receiving a blood meal prior to testing.

[3]Means followed by the same letter were not significantly different at the 5% level using Tukey's test (SAS 1994).

Table 3. Estimates of the difference in percentages of capture for male and female *Cimex lectularius* among furniture items contained in test arenas from simulated-field test in Richmond, CA. Contained in table are monitor type, location or furniture items, estimate of difference, standard error, degrees of freedom, t-value statistic, and p-values.

Monitor[1]	Location	Estimate of Difference	Std. Err.	DF	t-value	P-value
BDS	Rug	0.0810	0.0010	7	79.42	0.0000
BDS	Table	0.0647	0.0222	7	2.91	0.0227
Climbup	Monitor	0.0202	0.0002	7	99.00	0.0000
Climbup	Rug	0.0404	0.0004	7	99.02	0.0000
Control	Ground	0.2010	0.0664	7	3.03	0.0192
Control	Harborage	-0.2408	0.0629	7	-3.83	0.0065
Control	Table	0.0755	0.0226	7	3.35	0.0123
Nightwatch	Harborage	-0.0426	0.0025	5	-17.07	0.0000
Nightwatch	Monitor	0.0782	0.0222	5	3.52	0.0168

[1]BDS = Bedbug Detection System (passive) (Catchmaster™, AP&G Co., Inc., Brooklyn, NY), Climbup = Climbup® HD Insect Interceptor (Hotel Discreet; Susan McKnight, Inc., Memphis, TN) (passive), and Nightwatch = NightWatch™ (BioSensory, Inc., Putnam, CT) (active). For comparison, percentages for controls are included.

LIST OF FIGURES

of NightWatch™ showing felt-covered entry ramps and attached 680-g cylinder of compressed CO_2 gas and device display panel.

Figure 5. Wide view of the chambers built for the laboratory phase of monitor performance testing. There were three chambers. The vertical walls were constructed from clear polycarbonate plastic (Tap Plastics, Inc., El Cerrito, CA) and the base is made of Douglas-fir wood (Home Depot, El Cerrito, CA). The top cover is made of fine-nylon-mesh screen (Plankton Netting rated at 73 squares per inch; BioQuip, Gardena, CA). Also shown are small white lids covering bed bug harborages in each chamber prior to release. For details on how the walls were affixed to each other and wood floor see *Laboratory-testing arenas* in materials and methods.

Figure 6. Villa Termiti used for simulated-field testing on active and passive monitor performance. A) Exterior view showing entry door, warning sign, and one window sealed over with drywall. B) Interior view showing installation of new drywall and flooring.

Figure 7. Lay out of wooden testing arenas inside the Villa Termiti used for simulated-field tests of active and passive monitor performance. A) Wide-angle view of two wooden test arenas showing wagon-wheel alignment of furniture items and monitor undergoing testing. B) Close up of individual test arena showing vertical walls made of polycarbonate plastic, wooden floors made of two 2.4- by 2.4-m pieces of adjoining plywood sheets, L-brace supports, and long strips of adhesive tape on top of plastic walls to prevent bed bug escape. C) Close up view of a Climbup installed on the leg of the bed closest to the arena wall and in the opposite quadrant of the first monitor. For details on how the walls were affixed to each other and wood floor see *Constructing testing arenas* in materials and methods.

Figure 8. The frequency distribution of the bed bug, *Cimex lectularius*, adults found among five monitors. The number in proper area of monitor on the X-axis refers to groupings of bed bug counts found. The Y-axis contains nicknames for each monitor. White circles represent individual bed bugs and frequency (0 to 5) found in and around monitors. The vertical bar represented the mean number of bed

bugs found in and around individual monitors. The density of bed bugs tested included equal numbers of males (5) and females (5). For the untreated checks, there were no monitors in test arenas, thus the zero count frequency. BBALERT = BB Alert® Passive (MidMos Solutions Ltd., Brierly Hill, West Midlands, UK), BDS = Bedbug Detection System (BDS) (Catchmaster™, AP&G Co., Inc., Brooklyn, NY), ClimbUP = Climbup® Insect Interceptor (Susan McKnight, Inc., Memphis, TN), BBCatch = BB Catch™ (BioTrap™ Science, Portland, Oregon), and NightWatch = NightWatch™ (BioSensory, Inc., Putnam, CT). Laboratory tests conducted in Richmond, CA.

Figure 9. The mean percentages for *Cimex lectularius* adults found in furniture items and floor for each of five monitors. All bed bug densities (10, 50, 100) and replicates were combined to produce the traces presented. BDS = Bedbug Detection System (passive) (Catchmaster™, AP&G Co., Inc., Brooklyn, NY), Climbup = Climbup® HD Insect Interceptor (Hotel Discreet; Susan McKnight, Inc., Memphis, TN) (passive), and Nightwatch = NightWatch™ (BioSensory, Inc., Putnam, CT) (active). Simulated-field tests conducted in Richmond, CA.

Figure 10. Mean percentage of *Cimex lectularius* adults captured in all three monitors at three different bed bug densities (10, 50, 100). The white circles are actual data points and black horizontal lines are means for each density group tested. The 95% confidence interval for each mean is also depicted in the image. Simulated-field tests conducted in Richmond, CA.

Figure 11. Percentages of *Cimex lectularius* adults found in the interior floor square locations for three monitor products tested. Interior floor locations were defined as the four center squares based on a 16-square grid. The interior floor grids were also nearest the harborage release site and had at least one corner or leg for all furniture items placed in the test arenas. The black dots represent males and white dots females. The dark vertical bar represents the mean for each monitor and 95% confidence bands for means are also depicted in the image. The grey vertical hatched line is the 25% chance of bed bugs for the interior squares by chance along. For comparison, data points, mean, and 95% confidence band for

the control is also included. BDS = Bedbug Detection System (passive) (Catchmaster™, AP&G Co., Inc., Brooklyn, NY), Climbup = Climbup® HD Insect Interceptor (Hotel Discreet; Susan McKnight, Inc., Memphis, TN) (passive), and Nightwatch = NightWatch™ (BioSensory, Inc., Putnam, CT) (active). Simulated-field tests conducted in Richmond, CA.

Figure 12. Mean percentages of *Cimex lectularius* adults found among furniture items and the ground/floor surface when different monitors are deployed. The solid black line trace represents males, and the hatched line represent females. The smaller vertical light-grey lines represent significant differences (individual t-tests at a 5% significance level) among sexes. For comparison, the percentage by sex and furniture for the control is also presented. BDS = Bedbug Detection System (passive) (Catchmaster™, AP&G Co., Inc., Brooklyn, NY), Climbup = Climbup® HD Insect Interceptor (Hotel Discreet; Susan McKnight, Inc., Memphis, TN) (passive), and Nightwatch = NightWatch™ (BioSensory, Inc., Putnam, CT) (active). Simulated-field tests conducted in Richmond, CA.

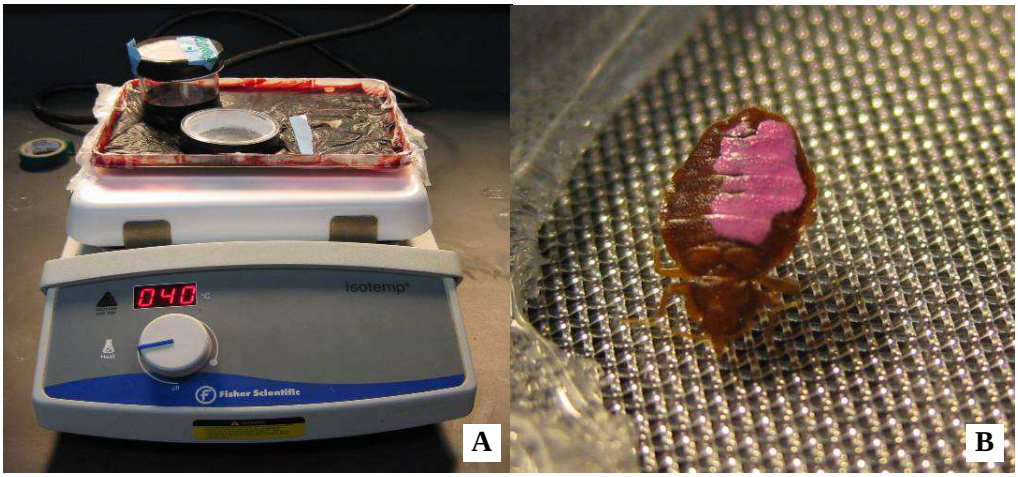
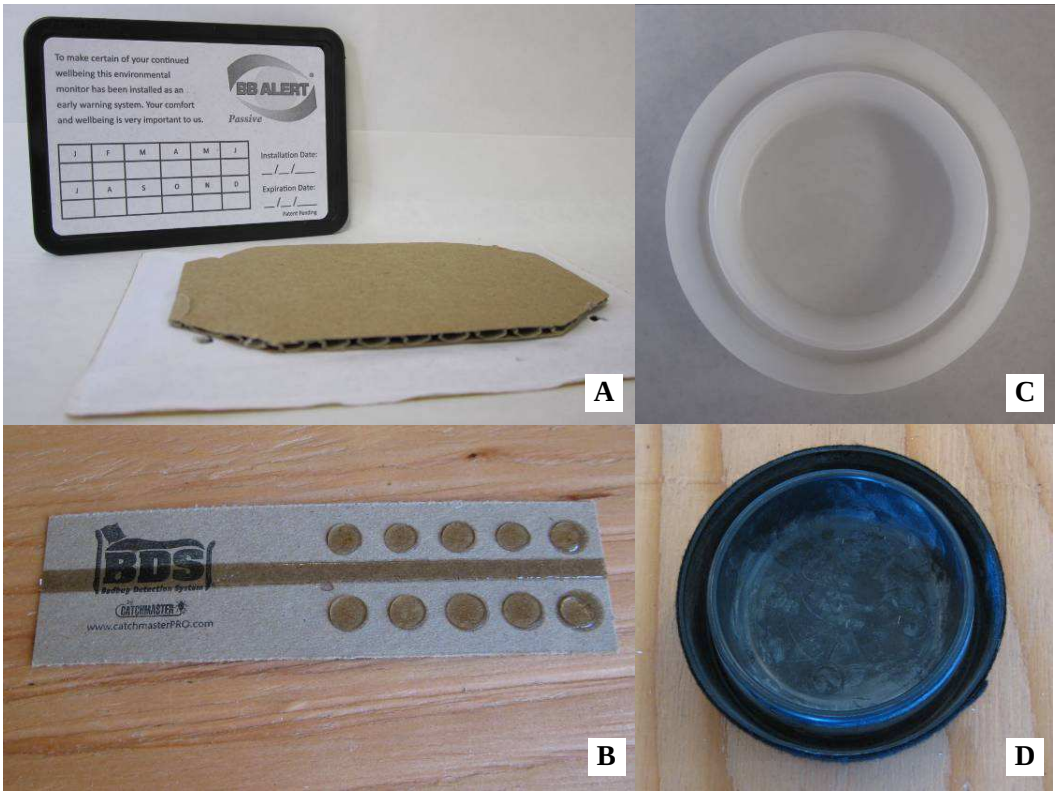


Figure 1

Figure 2



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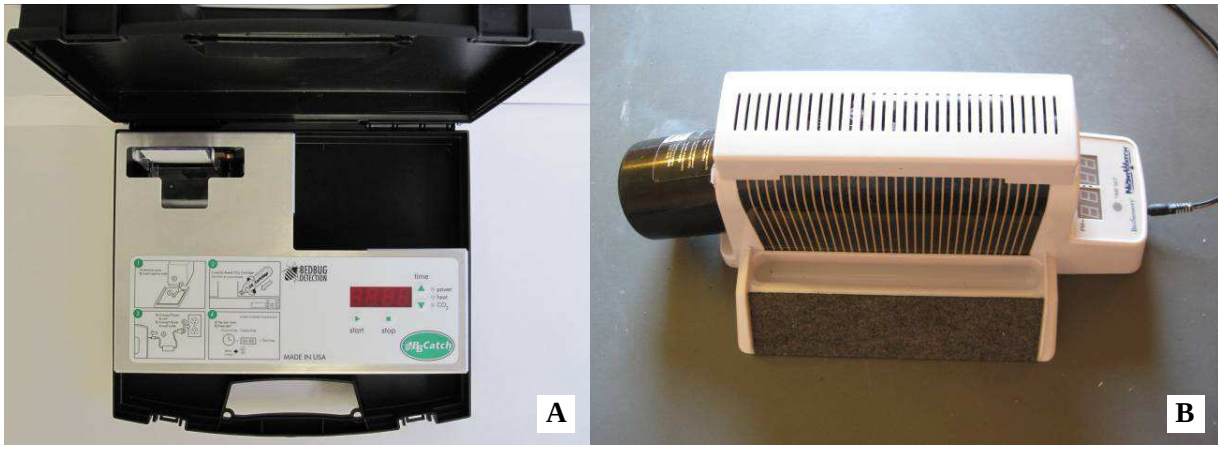

Figure 4

Figure 5

Figure 6

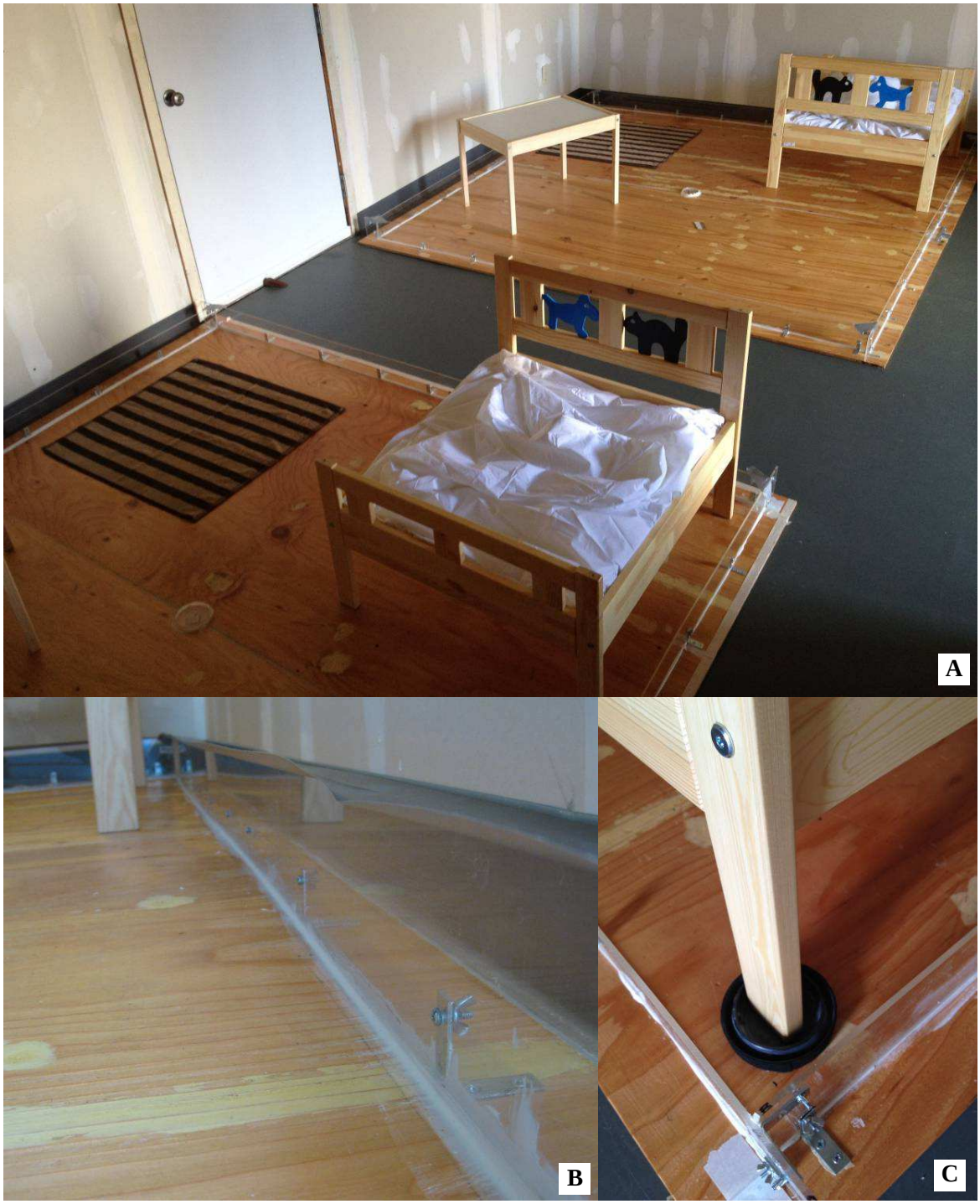

Figure 7

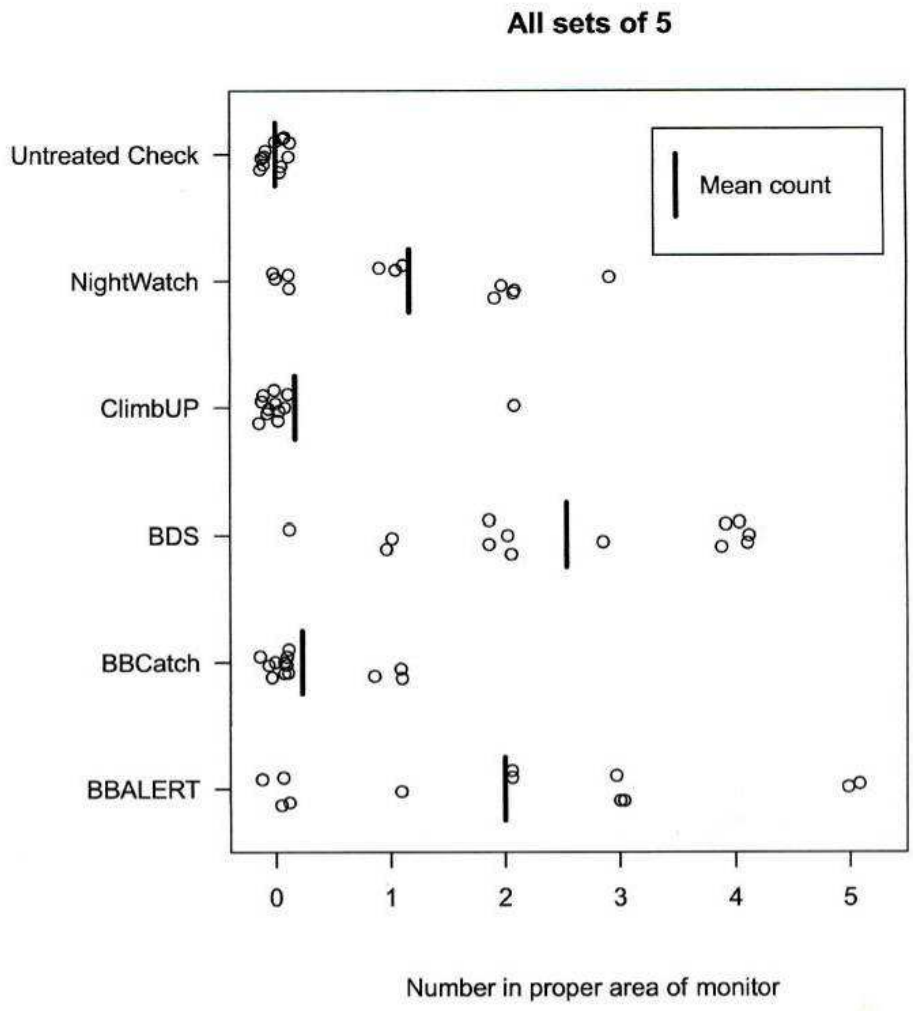
All sets of 5
Untreated Check
NightWatch
ClimbUP
BDS
BBCatch
BBALERT
Mean count
0
1
2
3
4
5
Number in proper area of monitor

Figure 8

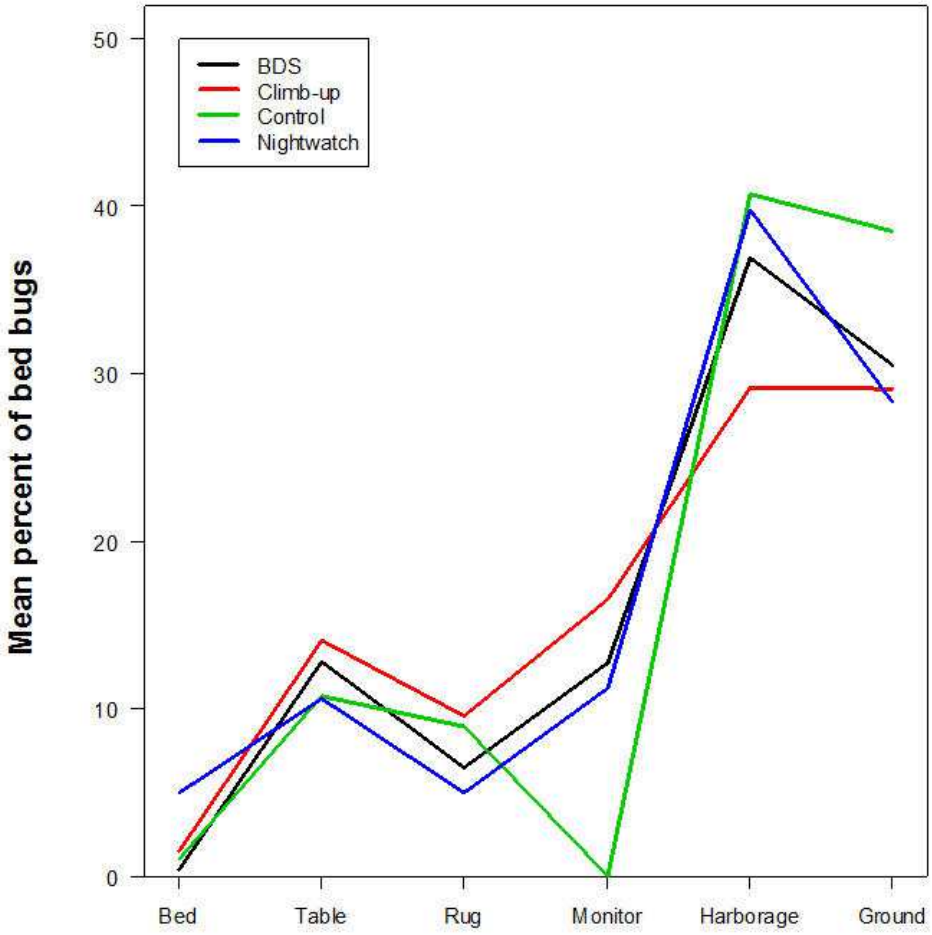
BDS
Climb-up
Control
Nightwatch
Mean percent of bed bugs
0
10
20
30
40
50
Bed
Table
Rug
Monitor
Harborage
Ground

Figure 9

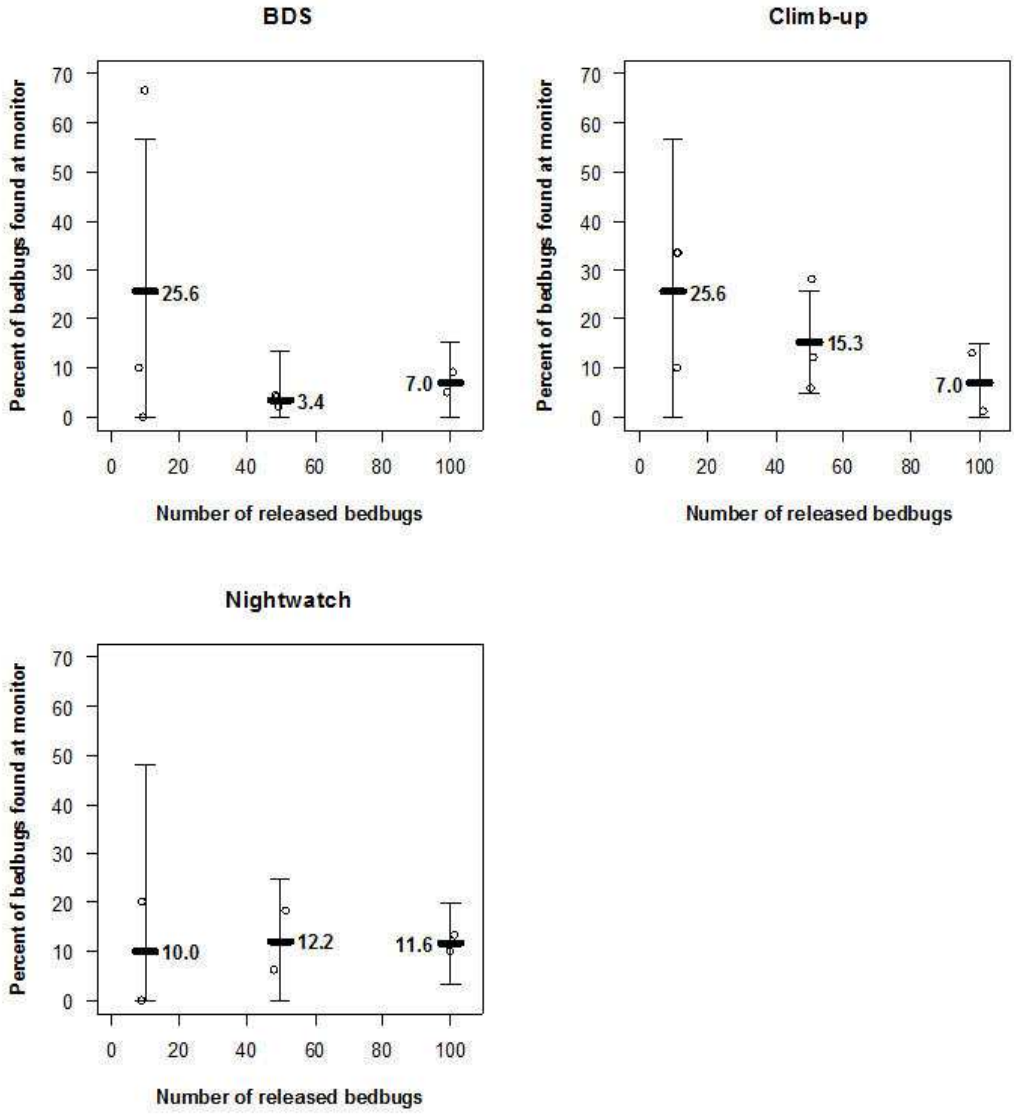
BDS
Percent of bedbugs found at monitor
Number of released bedbugs
25.6
3.4
7.0
Climb-up
Percent of bedbugs found at monitor
Number of released bedbugs
25.6
15.3
7.0
Nightwatch
Percent of bedbugs found at monitor
Number of released bedbugs
10.0
12.2
11.6

Figure 10

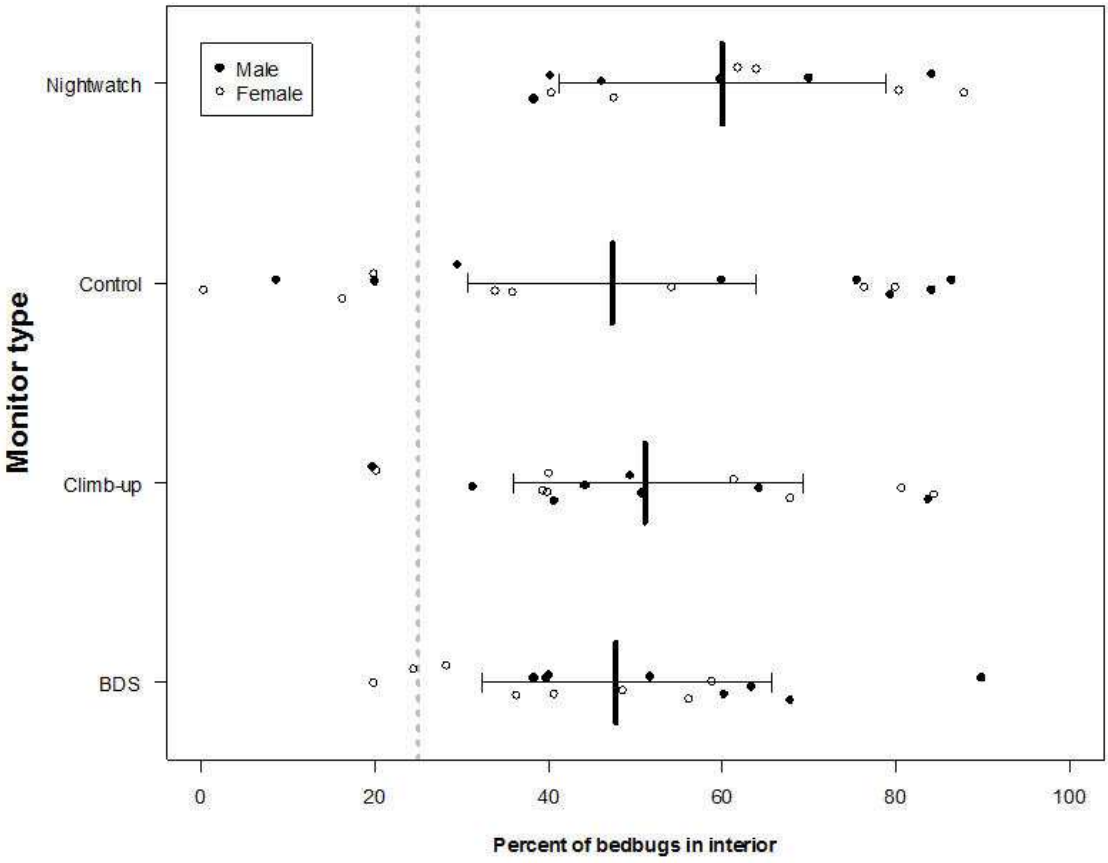
Male
Female
Nightwatch
Control
Climb-up
BDS
Monitor type
0
20
40
60
80
100
Percent of bedbugs in interior

Figure 11

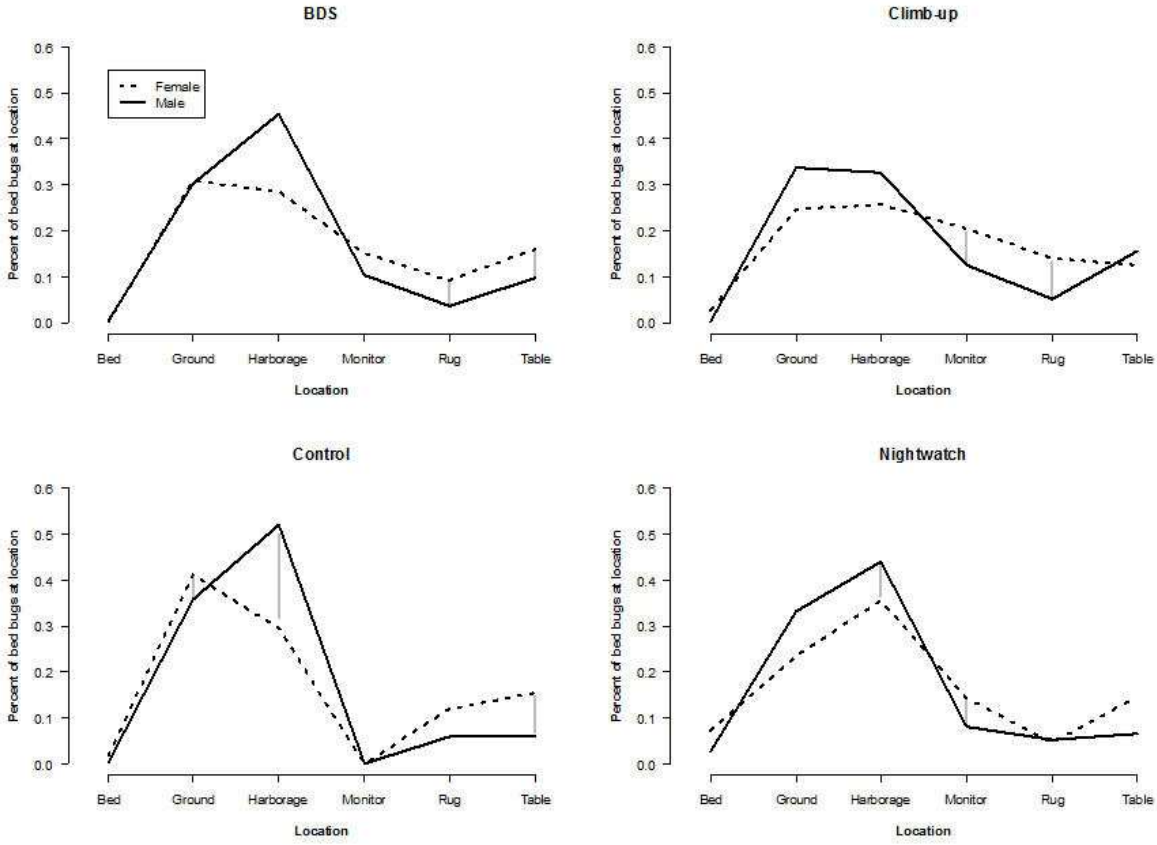
BDS
Climb-up
Control
Nightwatch
Female
Male
Percent of bed bugs at location
0.0
0.1
0.2
0.3
0.4
0.5
0.6
Bed
Ground
Harborage
Monitor
Rug
Table
Location

Figure 12