

Effect of Trap Design, Chemical Lure, Carbon Dioxide Release Rate, and Source of Carbon Dioxide on Efficacy of Bed Bug Monitors

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ABSTRACT Bed bugs, (*Cimex lectularius* L.), are difficult to find because of their nocturnal and secretive behavior. In recent years, a number of monitors containing carbon dioxide (CO₂), chemical lures, heat, or both, to attract bed bugs have been developed for detecting bed bugs. Ineffective trap design, lack of attraction of chemical lures, high cost of the CO₂ delivery system, or insufficient CO₂ release rates are some factors that limited the wide adoption of these monitors. To develop an affordable and effective monitor, we conducted a series of laboratory and field tests. Specifically, we tested a new pitfall trap design, a chemical lure mixture, different CO₂ release rates, and a sugar and yeast mixture as CO₂ source. Results show the new pitfall trap design was significantly more effective than Climbug insect interceptor, the most effective passive monitor available in the market for bed bugs. The experimental chemical lure mixture increased Climbug insect interceptor catch by 2.2 times. Results exhibit a distinct positive relationship between the CO₂ release rates and bed bug trap catches. There were no significant differences between CO₂ derived from cylinders and CO₂ generated from sugar and yeast mixture in their attractiveness to bed bugs. The findings suggest an effective and affordable monitor can be made incorporating the new pitfall trap design, a sugar and yeast mixture, and a chemical lure.

KEY WORDS bed bug, pitfall, carbon dioxide, chemical lure, sugar and yeast fermentation

In recent years, a number of active monitors have been developed in response to the resurgence of the bed bug, *Cimex lectularius* L. These active bed bug monitors incorporate carbon dioxide (CO₂), chemical lures, heat, or both, to attract and capture bed bugs foraging for bloodmeals or returning to a harborage site. Examples of such active monitors include CDC3000 (Cimex Science LLC, Portland, OR), Bed Bug Beacon (Nuvenco, Fort Collins, CO), Night-Watch (Biosensory Inc., Putnam, CT), First Response Bed Bug Monitor (SpringStar Inc., Woodinville, WA), and FMC Verifi (FMC Corporation, Philadelphia, PA) (Vaidyanathan and Feldlaufer 2013). Among these, only CDC3000, NightWatch, and Verifi have been found to be effective in detecting bed bugs (Wang et al. 2011; C.W. et al., unpublished data). CDC3000 and NightWatch are no longer produced because of their high cost, thus leaving Verifi as the only commercial active monitor that shows promise for bed bug detection. Trained dogs are another method for detection of bed bugs; however, results are inconsistent among detection teams, making the reliability of this method a concern (Wang and Cooper 2011). Other technologies used for detecting bed bug infestations include DNA analysis to distinguish bed bug fragments from other insects (Szalanski et al. 2011), gas chromatog-

raphy or mass spectrometry to identify airborne chemicals associated with bed bug infestations (Eom et al. 2011), and bed bug antigens or antigens from digested human blood present in bed bug feces (Smith 2010, Borth et al. 2011). The sophisticated and expensive nature of these technologies makes them impractical for widespread use. Moreover, their efficacy still needs to be measured under field conditions (Vaidyanathan and Feldlaufer 2013). Climbug insect interceptor (Susan McKnight Inc., Memphis, TN), referred to hereafter as “interceptor trap,” as a passive bed bug monitor is highly effective and has been used extensively for bed bug monitoring (Wang et al. 2010, 2011). However, this interception device is less effective in non-occupied environment where host cues are absent. There has been continued interest in developing affordable and reliable active bed bug monitors to help detect bed bugs early and measure treatment effectiveness.

Trap design can have a significant effect on bed bug trap efficacy. The effect of trap color (Strom and Goyer 2001, Roubos and Liburd 2008, Semeao et al. 2011), shape (Vernon and Gillespie 1995), size (Vernon and Gillespie 1995, Liburd et al. 1998), and texture of the outer surface (Hamilton et al. 1971, Granovsky 1983) on trap efficacy has already been demonstrated for other insect pests. Singh et al. (2012) conducted a series of laboratory experiments measuring the factors that may affect the efficacy of bed bug monitors. They found a chemical lure mixture consisting of nonanal,

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1-octen-3-ol, spearmint oil, and coriander Egyptian oil to be effective in attracting bed bugs. They did not find any significant relationship between CO₂ release rates (100, 200, 300, and 400 ml/min) and trap efficacy. However, it is not clear whether it is true under field conditions.

The cost of a CO₂ delivery system is a key factor determining cost of the active bed bug monitor. Gas cylinders (Hoel et al. 2011), dry ice (Wang et al. 2011), and a sugar and yeast fermenting mixture (Smallegange et al. 2010) have been used as a source of CO₂ for surveillance of hematophagous insects. Gas cylinders are expensive, cumbersome, and associated with risk of leakage. In addition, flow regulators are costly and sensitive to dust and high humidity (Mboera and Takken 1997, Saitoh et al. 2004). Dry ice can be difficult to obtain, transport, and store. In addition, dry ice can pose a hazard during handling and use (Xue et al. 2008). However, sugar and yeast fermentation method is convenient, cheap, and all the materials are locally available. Traps baited with sugar- and yeast-produced CO₂ has already been reported to be effective for monitoring different species of mosquitoes (Saitoh et al. 2004, Smallegange et al. 2010, Jawara et al. 2011) and the kissing bug, *Triatoma infestans* Klug (Guerenstein et al. 1995, Lorenzo et al. 1998). Sugar and yeast fermentation seems to have a great potential as a CO₂ delivery system in bed bug monitors.

Measuring the effect of different CO₂ release rates under field conditions will help in designing more effective bed bug monitors or improving the existing available monitors. In addition, a chemical lure with proven field efficacy may further maximize trap efficacy. An affordable and safe CO₂ source will lower the monitor cost and increase the acceptance and efficacy of bed bug monitors. The objectives of this study were 1) to determine the efficacy of a new pitfall trap design for monitoring bed bugs, 2) to determine whether a chemical lure mixture enhances bed bug trap catches under field conditions, 3) to measure the effect of different CO₂ release rates on trap efficacy, and 4) to determine whether CO₂ derived from gas cylinders and CO₂ generated from sugar and yeast fermenting mixture are equally effective for attracting bed bugs.

Materials and Methods

Insects for Laboratory Bioassays. Bed bugs were collected from an infested apartment in Newark, NJ, a few months before this study. They were maintained in plastic containers (4.7 cm in height and 5 cm in diameter) with folded paper as harborages at 26 ± 1°C, 40 ± 10% relative humidity (RH), and a photoperiod of 12:12 (L:D) h. They were fed weekly on defibrinated rabbit blood using a Hemotek membrane-feeding system (Discovery Workshops, Accrington, United Kingdom). Bugs were not fed for 2 wk before laboratory bioassays. Only males and large bed bug nymphs were used in this study. Females were not tested to avoid laying eggs in the arenas.

Field Site. All field experiments were conducted in occupied one-bedroom or studio apartments located

in Newark, NJ. Each one-bedroom apartment had a bedroom, living room, a kitchen, and a bathroom covering a total area of 56 m² (600 feet²). Each studio unit had a living room or bedroom, a kitchen, and a bathroom with a total area of 37 m² (400 feet²). Each apartment was occupied by one elderly person. These apartments were monitored biweekly at least for 4 wk using interceptor traps or visual inspections before the study.

Experiment 1. Effectiveness of a New Pitfall Trap Design for Trapping Bed Bugs. Laboratory Measurement. A new pitfall trap design was made with an inverted plastic dog bowl (600 ml of volume, 18 cm in diameter, 6.4 cm in depth) (IKEA, Baltimore, MD). The outer wall of the trap was covered with a layer of paper surgical tape (Caring International, Mundelein, IL), which was dyed black with Fiebing's Leather Dye (Tandy Leather Factory, Fort Worth, TX) (Fig. 1a). Interceptor traps were also dyed black and used for comparison with the new pitfall trap design. Bed bugs preferred black color to white color in our preliminary bioassays. The inside surfaces of both trap types were coated with a light layer of fluoropolymer resin (Bio-Quip products, Rancho Dominguez, CA) to prevent trapped bed bugs from escaping.

Plastic tray arenas (80 by 75 by 5 cm) (length by width by height) with bottom lined with brown paper were used (Fig. 1a). The brown paper was never changed during the entire study. A layer of fluoropolymer resin was applied to inner walls of the arenas to prevent the bugs from escaping. A filter paper (15 cm in diameter) was placed on the floor in the center of each arena, and then a plastic ring (13.3 cm in diameter and 6.4 cm in height) was placed on the filter paper for confining the bed bugs. A piece of folded cardboard and folded fabric was placed on the filter paper to provide harborages for bed bugs. Four additional bed bug-exposed paper harborages measuring 5.1 cm in length and 3.3 cm in width were placed along the edges of the floor of each tray arena. Four arenas were placed simultaneously in a nonventilated closed room measuring 4 m in length and 2.3 m in width at 24–25°C. A photoperiod of 12:12 (L:D) h was maintained in the room that was used for bioassays.

Two traps of the new pitfall trap design and two interceptor traps were placed at four corners of an arena equidistant (25 cm) from the center with each type placed diagonally opposite to each other (Fig. 1a). Fifty large nymphs and adult male bed bugs were released into the center of each arena and confined with a plastic ring. The bugs were acclimated for ≈15 h before the start of the experiment. At 1 h after dark cycle, CO₂ from a cylinder was released to the center of the room at 100 ml/min to stimulate bed bug activity. The plastic ring confining the bugs was removed. The numbers of bed bugs trapped in the pitfall traps and those in the arenas were collected and counted after 8 h with the aid of red light. After counting, dead and moribund bugs were replaced with healthy bugs in each arena. All bugs were placed back to the center of the arenas and confined with plastic



Fig. 1. Experimental set up for determining the efficacy of a new pitfall trap design: (a) laboratory test; and (b) field test. (Online figure in color.)

rings for 15 h before starting the next bioassay. Thus, each trap type was replicated 16 times over two consecutive days.

Field Measurement. The new pitfall trap design and interceptor traps were placed in pairs on the floor in bedrooms, living rooms, and bathrooms (Fig. 1b). The distances between the new pitfall trap design and interceptor traps were ≈ 24 cm. In total, 13 pairs were placed in two one-bed room apartments. The numbers of bed bugs caught in the traps were recorded after 14 d. Then the new pitfall trap and interceptor trap positions were switched, and the bed bug numbers inside the traps were recorded again after 14 d.

Experiment 2. Effect of a Chemical Lure Mixture on Trap Efficacy. Pairs of interceptor traps, ≈ 30 cm apart, were installed on floors adjacent to walls, floor

corners, and under the beds of two occupied apartments (Fig. 2). A chemical lure mixture consisting 25 μ l each of nonanal (Sigma-Aldrich Co., St. Louis, MO), 1-octen-3-ol, spearmint oil, and coriander Egyptian oil (Bedoukian Research Inc., Danbury, CT) was dispensed onto cotton within a 0.7-ml microcentrifuge tube. The lid of each tube had a 2-mm diameter opening to allow for slow release of the chemical vapor into the air. Within each interceptor trap pair, a microcentrifuge tube was placed at the center of one interceptor trap. The other interceptor trap did not receive any lure. Eleven pairs were placed in a one-bedroom apartment, and six pairs were placed in a studio apartment. The numbers of bed bugs caught in the interceptor traps were recorded after 2 d. Then the baited and unbaited interceptor trap positions were



Fig. 2. Climbug insect interceptor with and without chemical lure mixture. (Online figure in color.)

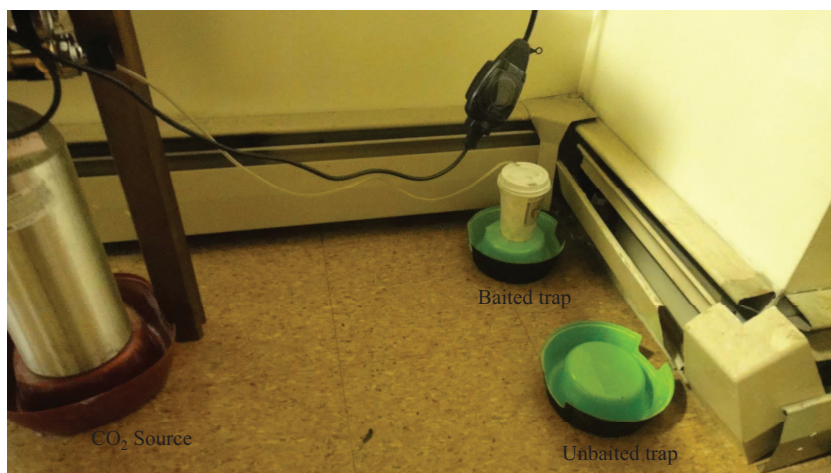


Fig. 3. Field set up to determine the effects of CO₂ release rates on trap efficacy. (Online figure in color.)

switched, and the bed bug numbers inside the interceptor traps were recorded after 6 d.

Experiment 3. Effect of CO₂ Release Rates on Trap Efficacy. The new pitfall trap design from experiment 1 was used to measure different CO₂ release rates. Pairs of traps, ≈60 cm apart, were placed adjacent to the sleeping areas of occupied apartments. Within each pair, one trap was supplied with 100% CO₂ from a 5 lb cylinder (Airgas East Inc., Piscataway, NJ), and the other trap was used as unbaited control. A CO₂ regulator (Milwaukee Instruments Inc., Rocky Mount, NC) was attached to each cylinder to control the release rate and time. The CO₂ release rate was determined as ml of bubble displaced by CO₂ per unit of time using a Bubble-O-Meter (Bubble-O-Meter, Dublin, OH). The CO₂ was introduced into 240-ml plastic cups that were placed on the traps (Fig. 3). CO₂ was released between 10:00 p.m. and 6:00 a.m.

Comparison Among 100, 200, and 400 ml/min. Three CO₂ release rates—100, 200, and 400 ml/min—were tested in three apartments. One rate was assigned to each apartment each day and then the rates were rotated among the three apartments for three consecutive days. Therefore, each apartment received three different rates over 3-d period. The numbers of bed bugs caught in the traps were recorded each day. The experiment was repeated 3 d later in the same manner to obtain a total of six replicates per CO₂ release rate.

Comparison Between 400 and 800 ml/min. The above test found 400 ml/min rate was significantly more effective than 100 and 200 ml/min rates. We then tested 400 and 800 ml/min rates in four apartments. Each CO₂ rate was assigned to two apartments on each day, and a different rate was used in each apartment the next day. The experiment was repeated on the third and fourth day, yielding a total of eight replicates per CO₂ release rate.

Experiment 4. Comparison of Two CO₂ Sources for Attracting Bed Bugs. CO₂ Sources. The two CO₂ sources tested were 5 lb cylinders and sugar and yeast mixture. In laboratory bioassays, the sugar and yeast

formulation tested consisted of 30 g yeast (Lesaffre Yeast Corporation, Milwaukee, WI), 150 g granulated cane sugar (U.S. Sugar Co. Inc., Buffalo, NY), and 1.5 liter warm water (40°C) in a 1-gallon plastic container. The mixture generated an average of 100 ml/min CO₂ for 4 h after initial mixing in a 25°C environment (Fig. 4). The rate was determined using a Bubble-O-Meter. In field experiments, the sugar and yeast formulation was 150 g yeast, 750 g granulated cane sugar, and 3 liter warm water in a plastic container (16 quart volume, 35 cm in length, 28 cm in width, 15.5 cm in height). The mixture generated an average of 400 ml/min CO₂ for 8 h after initial mixing (Fig. 4).

Laboratory Measurement. Large arenas (200 by 76 by 6.4 cm) (length by width by height) with a wooden floor were used. Two arenas were located in a 4-m-long and 2.3-m-wide room that had normal air current through vents on the ceilings. Two additional arenas were located in a nonventilated closed room measuring 4 m long and 2.3 m wide. A 25°C temperature and a photoperiod of 12:12 (L:D) h was maintained in both rooms. The new pitfall trap design from experiment 1 was used. Each arena had an unbaited control trap and a trap baited with CO₂. The two traps were placed at opposite ends equidistant (85 cm) from the center. In each room, one arena was used to test CO₂ from cylinder at 100 ml/min (Fig. 5a) and the other arena was used to test CO₂ from sugar and yeast mixture, which released CO₂ at an average rate of 100 ml/min for 4 h (Fig. 5b). Six additional bed bug-exposed paper harborage measuring 5.1 cm in length and 3.3 cm in width were placed along the edges of the floor of each wooden arena. Seventy nymphs and adult bed bug males were released into the center of each arena. The numbers of bed bugs trapped in the traps and those in the arenas were collected and counted after 4 h with the aid of red light. A 4-h period has been observed to be sufficient for observing the effect of CO₂ in preliminary bioassays. All other testing procedures including confining, handling, and collecting bed bugs were similar to experiment 1. The experiment was

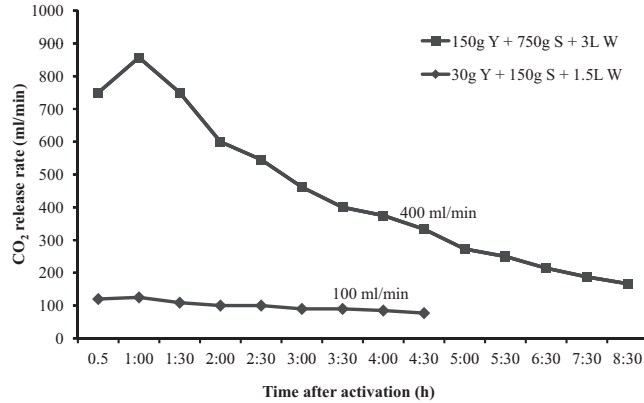


Fig. 4. CO₂ release rates from sugar–yeast–water mixture starting from 30 min after mixing. Y, yeast; S, sugar; W, water. Warm water (40°C) was added to sugar and yeast mixture and stirred for 5 min. The room temperature was 25°C.

repeated four times over four consecutive days to obtain eight replicates per treatment. The baited and unbaited trap positions in each arena were switched after 2 d.

Field Measurement. Five one-bedroom apartments and one studio apartment were used. All apartments had low numbers of bed bugs based on our biweekly monitoring using interceptor traps and visual inspections. Both bedrooms and living rooms were used in four of the one-bedroom apartments. In the other two apartments, only the bedrooms were used. During each night, one monitor was placed in each room. When sugar and yeast mixture was used, two pitfall traps were placed under the sugary–yeast box for catching bed bugs (Fig. 6a). When CO₂ cylinders were used, two pitfall traps were deployed 24 cm apart in a similar fashion as the sugar and yeast mixture set up. One trap was baited with CO₂ at 400 ml/min and the other one was unbaited (Fig. 6b). The total count from the two traps in each room after one night deployment was used to compare the two types of CO₂ sources. In the first night, five rooms were used to test CO₂ derived from cylinders and the other five rooms

were used to test CO₂ generated from the sugar and yeast mixture. Then the type of CO₂ source was switched in each room on the second night, providing 10 replicates per CO₂ source. The CO₂ cylinders were set to release for 8 h (10:00 p.m. to 6:00 a.m.) each night. The sugar and yeast mixture released CO₂ continuously on each day immediately after set up (between 6:00 and 8:00 p.m.) until the traps were taken down (≈24 h). In addition to CO₂, a 0.7-ml microcentrifuge tube containing chemical lure mixture described in experiment 2 was placed on top of the pitfall trap that received CO₂ from a cylinder or on top of the container holding the sugar and yeast mixture.

Statistical Analyses. Field Tests. Bed bug numbers captured in traps were logarithmic transformed to meet assumptions of normality and homogeneity of variance (Zar 1999). A paired *t*-test (*P* = 0.05) was used: 1) to compare the bed bug counts in the new pitfall trap design and interceptor traps for each sampling date, and 2) to compare the bed bug counts in interceptor traps baited with chemical lure mixture and unbaited interceptors for each sampling date. Analysis of variance (ANOVA) was conducted to

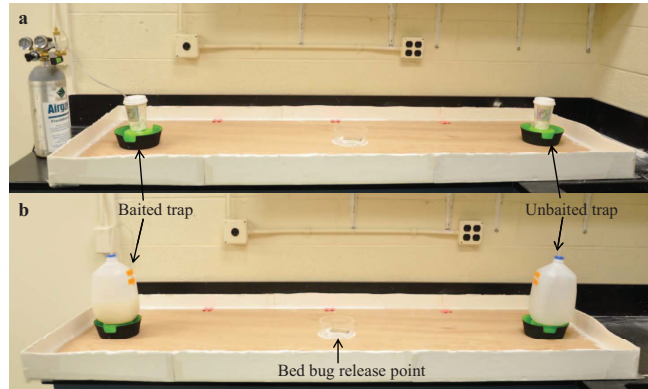


Fig. 5. Laboratory set up for determining the attractiveness of two CO₂ sources to bed bugs: (a) a large “wooden door” arena with a pitfall trap baited with CO₂ derived from a cylinder and an unbaited trap; and (b) a wooden door arena with a pitfall trap baited with CO₂ derived from sugar–yeast–water mixture and an unbaited trap. (Online figure in color.)



Fig. 6. Field set up for comparing the attractiveness of two CO₂ sources: (a) sugar–yeast–water mixture; and (b) a 5-lb CO₂ cylinder equipped with a regulator. (Online figure in color.)

compare the bed bug counts among different CO₂ release rates and between two CO₂ sources. Means were separated using Tukey's honestly significant difference (HSD) test ($P = 0.05$). All analyses were conducted using SAS software (SAS Institute 2009).

Laboratory Bioassays. Bed bug distribution among traps in each arena was summarized as percentage of bed bugs in traps and percentage of bugs remained in the arena. Generalized mixed linear models (PROC GLIMMIX) were used to analyze the trap counts. The model accommodates random effects (cohort), repeated measures, and overdispersion. In all experiments, only those bed bugs that appeared in the traps were analyzed. Those bugs that remained in the arenas at the end of the experiments were weak, inactive, or behaviorally different from those actively seeking for a host. Previous observations indicate that the presence of bed bugs in a trap had no significant effect on the probability of trapping additional bed bugs. Therefore, the bed bugs in the traps were considered inde-

pendent events and were not related to gregarious behavior.

Results

Effectiveness of a New Pitfall Trap Design for Trapping Bed Bugs. In laboratory bioassays, the new pitfall trap design caught significantly more bed bugs than the interceptor trap ($F = 10.64$; $df = 1$; $P = 0.0001$). The probability of trapping bed bugs in new pitfall trap design and interceptor trap was 77.2 ± 2.1 and $22.8 \pm 2.1\%$, respectively. In field test, the new pitfall trap design caught 2.6- and 3.2-fold more bed bugs than the interceptor trap during 0–14 d ($t = 5.02$; $df = 12$; $P = 0.0003$) and 15–28 d ($t = 5.82$; $df = 12$; $P = 0.0001$), respectively. Overall, the new pitfall trap design caught significantly more (2.8-fold) bed bugs than the interceptor trap ($t = 7.74$; $df = 26$; $P = 0.0001$) (Fig. 7).

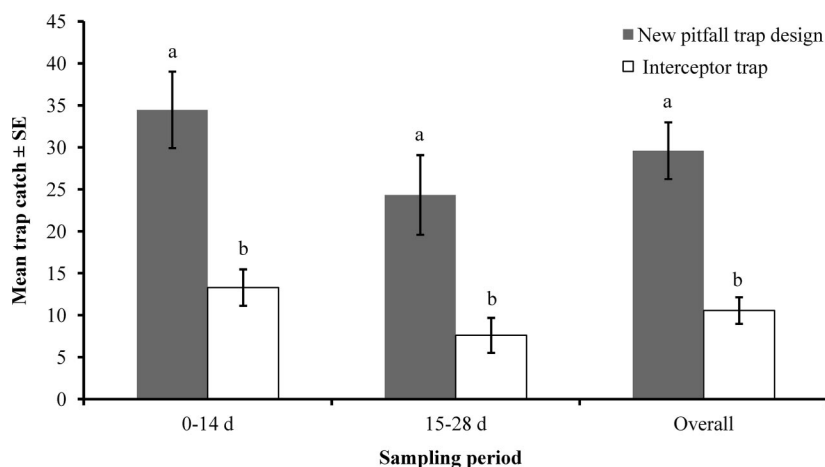


Fig. 7. Comparison of two traps in apartments. Within the same sampling period, bars with different letters are significantly different ($P < 0.05$; paired t -test). Analysis was based on logarithmic transformed data, but actual mean values are shown.

Effect of a Chemical Lure Mixture on Trap Efficacy. Interceptor traps baited with a chemical lure mixture caught significantly greater number of bed bugs than the unbaited interceptors during 0–2 d ($t = 2.26$; $df = 16$; $P = 0.04$) and 3–8 d ($t = 3.86$; $df = 15$; $P = 0.002$). The baited interceptors caught an average of 2.2-fold more bed bugs than the unbaited interceptors during 0–2 d. During 3–8 d, the baited interceptors caught an average of 2.3-fold more bed bugs than the unbaited interceptors (Fig. 8).

Effect of CO₂ Release Rates on Trap Efficacy. Traps baited with CO₂ caught much higher numbers of bed bugs than their corresponding unbaited traps, indicating the importance of CO₂ for trapping bed bugs ($P < 0.05$) (Fig. 9). There were significant differences among 100, 200, and 400 ml/min ($F = 5.40$; $df = 2$; $P = 0.03$) and between 400 and 800 ml/min ($F = 6.52$; $df = 1$; $P = 0.02$) in their effect on trap efficacy. The 400 ml/min rate was significantly more effective than 100 ml/min ($P < 0.05$) (Fig. 9a), and

800 ml/min was significantly more effective than 400 ml/min (Fig. 9b).

Comparison of Two CO₂ Sources for Attracting Bed Bugs. There were no significant differences between CO₂ derived from cylinders and sugar- and yeast-generated CO₂ in their attractiveness to bed bugs in laboratory bioassays ($F = 0.29$; $df = 1$; $P = 0.60$). The probability of bed bugs being caught in traps baited with CO₂ from cylinders and sugar- and yeast-generated CO₂ was 90.0 ± 1.4 and $91.0 \pm 1.3\%$, respectively. Similarly, tests in apartments did not show significant differences in trap counts between the two CO₂ sources ($F = 0.23$; $df = 1$; $P = 0.64$) (Fig. 10).

Discussion

Currently, interceptor traps are the most effective passive bed bug monitors for detecting bed bugs, and proved to be more effective than visual inspections (Wang et al. 2009, 2011). Results from this study in-

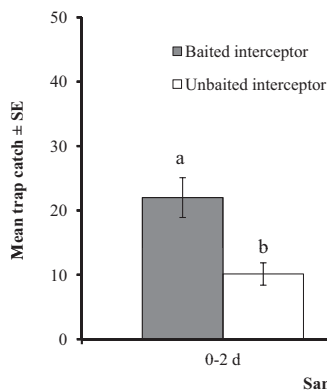


Fig. 8. Effect of a chemical lure mixture on trap catches in apartments. Within the same sampling period, bars with different letters are significantly different ($P < 0.05$; paired t -test). Analysis was based on logarithmic transformed data, but actual mean values are shown.

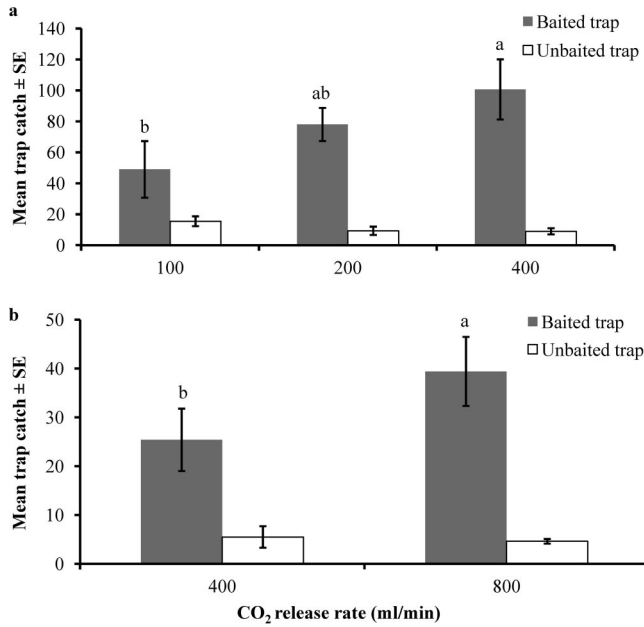


Fig. 9. Effect of CO₂ release rates on trap efficacy in apartments: (a) 100, 200, and 400 ml/min; and (b) 400 and 800 ml/min. Bars with different letters are significantly different ($P < 0.05$; Tukey's HSD test). Analysis was based on logarithmic transformed data, but actual mean values are shown.

dicating that the interceptor traps can be improved by increasing the depth of the trap. We frequently found that bed bugs fall into the outer well of the interceptor traps and could escape into the inner well under field conditions. It is reasonable to believe that a significant number of bugs would also be able to escape from the interceptor once trapped. The new pitfall trap design was much taller than the interceptor trap and made it more difficult for bed bugs to escape. Its effectiveness can be further enhanced by adding attractants such as carbon dioxide, chemical lure, or heat. This trap can be placed anywhere inside the home to detect bed bug infestations, to determine the

distributions of bed bugs in an infested dwelling, and to measure treatment results. This monitor may also be combined with insecticides to attract and kill bed bugs that are attracted to the monitor.

The chemical lure mixture measured in this study is the first with proven efficacy in field studies. Field results corroborated our laboratory findings, that the chemical lure mixture is attractive to bed bugs and can significantly enhance the effectiveness of a bed bug monitor (Singh et al. 2012). The chemical lure mixture may also be used in conjunction with other types of bed bug-trapping devices by simply placing the lure on top or inside the trapping devices.

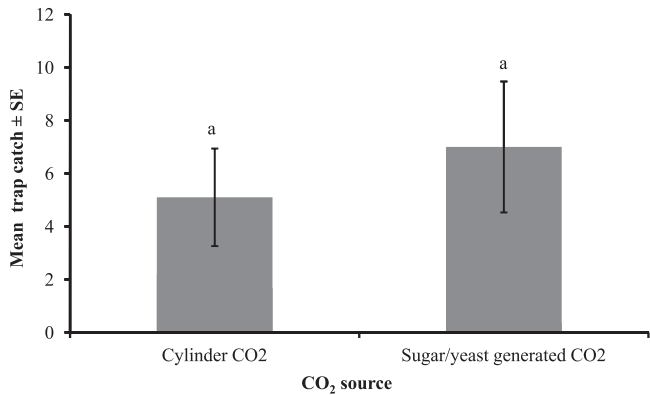


Fig. 10. Comparison of two CO₂ sources for attracting bed bugs in occupied apartments after 1 d placement. The sugar-yeast-water mixture was 150 g yeast, 750 g granulated cane sugar, and 3 liter warm (40°C) water. The CO₂ cylinder released CO₂ at 400 ml/min. Bars with same letters are not significantly different ($P > 0.05$; Tukey's HSD test). Analysis was based on logarithmic transformed data, but actual mean values are shown.

Our previous study indicates various CO₂ release rates (100, 200, 300, and 400 ml/min) yielded similar trap catches when tested in large arenas (2 m in length) (Singh et al. 2012). However, results from this study suggest a distinct positive relationship between the CO₂ release rates (100 vs. 400 vs. 800 ml/min) and bed bug trap catches under field conditions. The discrepancy between laboratory and field results may be because of the fact that human hosts were present during the trapping period in apartments. It is possible that to compete with the natural host, monitors need to use CO₂ release rates higher than the human respiration rate (250 ml/min for an adult human) (Leff and Schumacker 1993). In addition, a higher CO₂ release rate will also be necessary to overcome the effect of larger space, air movement, various odors from human hosts, as well as physical obstacles such as clutter present in a room. We did not test rates above 800 ml/min because of potential health risks associated with elevated CO₂ levels.

Similar relationships between CO₂ release rates and efficacy have been found in mosquitoes. A higher CO₂ release rate has been shown to increase trap catch and range of attractiveness under field conditions (McIver and McElligott 1989, Kline et al. 1991, Dekkar and Takken 1998). Sugar and yeast baited traps with a flow rate of 136 ml/min caught significantly fewer mosquitoes than traps baited with sugar and yeast that generated a CO₂ at flow rate of 303 ml/min (Smallegange et al. 2010).

Our study clearly demonstrates that a sugar and yeast mixture can replace CO₂ cylinders as the CO₂ source. CO₂ was continuously generated from the sugar and yeast mixture for at least 24 h, but the release rates declined over the time. The release rates can be controlled by adjusting the amount of yeast, sugar, and water. A high CO₂ release rate equivalent to that from a CO₂ cylinder can be generated at an affordable cost. The materials needed for sugar and yeast CO₂ generation are inexpensive, safe, and do not require special training. Disadvantages of the sugar and yeast fermentation method are that a large container is needed for generating sufficient CO₂ release rate and the release rate diminishes over time. Despite these disadvantages, sugar and yeast fermentation appears to be a promising alternative for CO₂ from gas cylinders. It is a safe and affordable alternative for home owners. The trapping system incorporating sugar and yeast, chemical lure, and an effective pitfall trap can be especially useful for detecting bed bugs in vacant rooms and nontraditional locations such as schools, hospitals, offices, theaters, and so forth.

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References Cited

- Borth, P. W., N. Orr, P. N. Scherer, B. M. Schneider, M. P. Tolley, C. J. Voglewede, G. D. Crouse, D. G. McCaskill, K. Y. Yau, E. L. Olberding, et al. 2011. Bed bug detection, monitoring and control techniques. U.S. patent 7,743,552.
- Dekkar, T., and W. Takken. 1998. Differential responses of mosquito sibling species *Anopheles arabiensis* and *An. Quadriannulatus* to carbon dioxide, a man or a calf. *Med. Vet. Entomol.* 12: 136–140.
- Eom, I. Y., S. Risticvic, and J. Pawliszyn. 2011. Simultaneous sampling and analysis of indoor air infested with *Cimex lectularius* L. (Hemiptera: Cimicidae) by solid phase microextraction, thin film microextraction and needle trap device. *Anal. Chim. Acta.* 716: 2–10.
- Granovsky, T. A. 1983. Effect of exterior surface texture on cockroach jar trap efficacy. *Environ. Entomol.* 12: 744–747.
- Guerenstein, P. G., M. G. Lorenzo, J. A. Nffiez, and C. R. Lazzari. 1995. Baker's yeast, an attractant for baiting traps for Chagas' disease vectors. *Experientia* 51: 834–837.
- Hamilton, D. W., P. H. Schwartz, B. G. Townshend, and C. W. Jester. 1971. Effect of colour and design of traps on captures of Japanese beetles and bumblebees. *J. Econ. Entomol.* 64: 430–432.
- Hoel, D. F., G. E. Zollner, S. S. El-Hossary, E. Y. Fawaz, N. Watany, H. A. Hanafi, P. J. Obenauer, and P. Kirsch. 2011. Comparison of three carbon dioxide sources on phlebotomine sand fly capture in Egypt. *J. Med. Entomol.* 48: 1057–1061.
- Jawara, M., T. S. Awolola, M. Pinder, D. Jeffries, and R. C. Smallegange. 2011. Field testing of different chemical combinations as odour baits for trapping wild mosquitoes in the Gambia. *PLoS ONE* 6: e19676.
- Kline, D. L., D. A. Dame, and M. V. Meisch. 1991. Evaluation of 1-octen-3-ol and carbon dioxide as attractants for mosquitoes associated with irrigated rice fields in Arkansas. *J. Am. Mosq. Control Assoc.* 7: 165–169.
- Leff, A. R., and P. T. Schumacker. 1993. Respiratory physiology basics and applications. W. B. Saunders Co., Philadelphia, PA.
- Liburd, O. E., S. R. Alm, R. A. Casagrande, and S. Polavarapu. 1998. Effect of trap color, bait, shape, and orientation in attraction of blueberry maggot (Diptera: Tephritidae) flies. *J. Econ. Entomol.* 91: 243–249.
- Lorenzo, M. G., C. E. Reisenman, and C. R. Lazzari. 1998. *Triatoma infestans* can be captured under natural climatic conditions using yeast-baited traps. *Acta Trop.* 70: 277–284.
- Mboera, L.E.G., and W. Takken. 1997. Carbon dioxide chemotaxis in mosquitoes (Diptera: Culicidae) and its potential in vector surveillance and management programmes. *Rev. Med. Vet. Entomol.* 85: 355–368.
- McIver, S. B., and P. E. McElligott. 1989. Effects of release rates on the range of attraction of carbon dioxide to some southwestern Ontario mosquito species. *J. Am. Mosq. Control Assoc.* 5: 6–9.
- Roubos, C. R., and O. E. Liburd. 2008. Effect of trap color on captures of grape root borer (Lepidoptera: Sesiidae) males and non-target insects. *J. Agric. Entomol.* 25: 99–109.
- Saitoh, Y., J. Hattori, S. Chinone, N. Nihei, Y. Tsuda, H. Kura-hashii, and M. Kobayashi. 2004. Yeast-generated CO₂ as a convenient source of carbon dioxide for adult mosquito sampling. *J. Am. Mosq. Control Assoc.* 20: 261–264.

- SAS Institute. 2009. SAS/STAT user's guide, version 9.2. SAS Institute, Cary, NC.
- Semeao, A. A., J. F. Campbell, R. J. Whitworth, and P. E. Sloderbeck. 2011. Response of *Tribolium castaneum* and *Tribolium confusum* adults to vertical black shapes and its potential to improve trap capture. *J. Stored Prod. Res.* 47: 88–94.
- Singh, N., C. Wang, R. Cooper, and C. Liu. 2012. Interactions among carbon dioxide, heat, and chemical lures in attracting the bed bug, *Cimex lectularius* L. (Hemiptera: Cimicidae). *Psyche* 2012: 1–9. doi:10.1155/2012/273613.
- Smallegange, R. C., W. H. Schmied, K. J. Van Roey, N. O. Verhulst, J. Spitzen, W. R. Mukabana, and W. Takken. 2010. Sugar-fermenting yeast as an organic source of carbon dioxide to attract the malaria mosquito *Anopheles gambiae*. *Malar. J.* 9: 292.
- Smith, N. L. 2010. Detection and measurement of blood-feeding activity. U.S. patent 7,727,734.
- Strom, B. L., and R. A. Goyer. 2001. Effect of silhouette color on trap catches of *Dendroctonus frontalis* (Coleoptera: Scolytidae). *Ann. Entomol. Soc. Am.* 94: 948–953.
- Szalanski, A. L., A. D. Tripodi, and J. W. Austin. 2011. Multiplex polymerase chain reaction diagnostics of bed bug (Hemiptera: Cimicidae). *J. Med. Entomol.* 48: 937–940.
- Vaidyanathan, R., and M. F. Feldlaufer. 2013. Bed bug detection: current technologies and future directions. *Am. J. Trop. Med. Hyg.* 88: 619–625.
- Vernon, R. S., and D. R. Gillespie. 1995. Influence of trap shape, size and background color on captures of *Frankliniella occidentalis* (Thysanoptera: Thripidae) in a cucumber greenhouse. *J. Econ. Entomol.* 88: 288–293.
- Wang, C., T. J. Gibb, and G. W. Bennett. 2009. Interceptors assist in bed bug monitoring. *Pest Control Technol.* 37: 112, 114.
- Wang, C., K. Saltzmann, E. Chin, G. W. Bennett, and T. Gibb. 2010. Characteristics of *Cimex lectularius* (Hemiptera: Cimicidae) infestation and dispersal in a high-rise apartment building. *J. Econ. Entomol.* 103: 172–177.
- Wang, C., W. Tsai, R. Cooper, and J. White. 2011. Effectiveness of bed bug monitors for detecting and trapping bed bugs in apartments. *J. Econ. Entomol.* 104: 274–278.
- Wang, C., and R. Cooper. 2011. Detection tools and technologies. *Pest Control Technol.* 39: 72, 74, 76, 78–79, 112.
- Xue, R. D., M. A. Doyle, and D. L. Kline. 2008. Field evaluation of CDC and Mosquito Magnet X traps baited with dry ice, CO₂ sachet, and octenol against mosquitoes. *J. Am. Mosq. Control Assoc.* 24: 249–252.
- Zar, J. H. 1999. Biostatistical analysis, 4th ed. Prentice Hall, Upper Saddle River, NJ.

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