Journal of Economic Entomology Advance Access published March 22, 2015

Effectiveness of a Sugar–Yeast Monitor and a Chemical Lure for Detecting Bed Bugs

NARINDERPAL SINGH, CHANGLU WANG[1], AND RICHARD COOPER

Department of Entomology, Rutgers University, 93 Lipman Drive, New Brunswick, NJ 08901

J. Econ. Entomol. 1–6 (2015); DOI: 10.1093/jee/tov061

ABSTRACT Effective bed bug (*Cimex lectularius* L.) monitors have been actively sought in the past few years to help detect bed bugs and measure the effectiveness of treatments. Most of the available active monitors are either expensive or ineffective. We designed a simple and affordable active bed bug monitor that uses sugar–yeast fermentation and an experimental chemical lure to detect bed bugs. The sugar–yeast mixture released carbon dioxide at a similar rate (average 405.1 ml/min) as dry ice (average 397.0 ml/min) during the first 8 h after activation. In naturally infested apartments, the sugar–yeast monitor containing an experimental chemical lure (nonanal, L-lactic acid, 1-octen-3-ol, and spearmint oil) was equally effective as the dry ice monitor containing the same lure in trapping bed bugs. Placing one sugar–yeast monitor per apartment for 1-d was equally effective as 11-d placement of 6–18 Climbup insect interceptors (a commonly used bed bug monitor) under furniture legs for trapping bed bugs. When carbon dioxide was present, pair-wise comparisons showed the experimental lure increased trap catch by 7.2 times. This sugar–yeast monitor with a chemical lure is an affordable and effective tool for monitoring bed bugs. This monitor is especially useful for monitoring bed bugs where a human host is not present.

KEY WORDS sugar–yeast, dry ice, carbon dioxide, chemical lure

Effective bed bug (*Cimex lectularius* L.) monitors have been actively sought in the past few years to help detect bed bugs early, guide treatments to target areas, and measure the effectiveness of treatments. Passive bed bug monitors (monitors that do not contain lures) such as Climbup insect interceptors (Susan McKnight Inc., Memphis, TN) referred to hereafter as interceptors are used extensively for bed bug monitoring (Wang et al. 2009, 2011). Drawbacks of passive monitors include heavy lifting of furniture, up to 14-d placement to confirm the presence of bed bugs, and reduced effectiveness in nonoccupied environments. As a result, there has been continued interest in developing active bed bug monitors that use carbon dioxide (CO_2), chemical lure, and heat for attracting bed bugs in both occupied and nonoccupied environments.

CO_2 release rate is the determining factor in the efficacy of an active bed bug monitor. There is a distinct positive relationship between the CO_2 release rates and bed bug trap catches (Singh et al. 2013). For example, a dry ice monitor was found to be more effective than two commercially available active monitors (CDC 3000 and NightWatch) in bed bug-infested apartments (Wang et al. 2011), primarily due to the dry ice monitor's high CO_2 release rate (731 ml/min) compared with CDC 3000 (42 ml/min) and NightWatch (161 ml/min). Similar relationships between CO_2 release rate and trap efficacy have been found in mosquitoes (McIver and McElligott 1989, Kline et al. 1991, Dekkar and Takken 1998). Sugar–yeast traps with a release rate of 136 ml/min CO_2 caught significantly fewer mosquitoes than the traps with 303 ml/min CO_2 (Smallegange et al. 2010).

In order to compete with the human host in an occupied environment, an active bed bug monitor may need to release CO_2 at a rate that is competitive to the human respiration rate of 250 ml/min (Leff and Schumacker 1993). With the exception of the NightWatch monitor, commercially available active monitors such as Bed Bug Beacon (Nuvenco, Fort Collins, CO), Verifi (FMC Corporation, Philadelphia, PA), First Response Bed Bug Monitor (SpringStar Inc., Woodinville, WA), etc. produce <50 ml/min CO_2, which is much lower than the human respiration rate. The insufficient CO_2 release rates render these monitors either ineffective or have very limited effective range, therefore requiring multiple monitors to be installed per room.

Gas cylinders (Hoel et al. 2011, Jawara et al. 2011), dry ice (Russell 2004, Oli et al. 2005, Hoel et al. 2011, Wang et al. 2011), and a sugar–yeast fermenting mixture (Oli et al. 2005, Smallegange et al. 2010) have been used as CO_2 sources for surveillance of hematophagus insects. Gas cylinders are expensive, cumbersome, and associated with risk of leakage (Saitoh et al. 2004). Dry ice can be difficult to obtain, transport, and store, and can pose a hazard during handling and use (Oli et al. 2005, Xue et al. 2008). On the other hand, sugar–yeast fermentation method is convenient, cheap, and all the materials are readily available. Sugar–yeast

[1] Corresponding author, e-mail: cwang@aesop.rutgers.edu.

© The Authors 2015. Published by Oxford University Press on behalf of Entomological Society of America. All rights reserved. For Permissions, please email: journals.permissions@oup.com

baited traps have been shown to be effective for monitoring different species of mosquitoes (Saitoh et al. 2004, Smallegange et al. 2010) and the kissing bug, *Triatoma infestans* Klug (Lorenzo et al. 1998). Sugar–yeast baited monitors have been shown to be equally effective as CO_2 cylinder baited monitors for trapping bed bugs in low-level infested apartments (Singh et al. 2013). However, the effectiveness of the sugar–yeast monitor has not been evaluated against the most effective active monitor (dry ice monitor; Wang et al. 2011) and the most widely used passive bed bug monitor (Climbup insect interceptor; Wang et al. 2009, 2011).

Beside CO_2, a chemical lure mixture consisting of nonanal, 1-octen-3-ol, spearmint oil, and coriander Egyptian oil was found effective in attracting bed bugs (Singh et al. 2013). Its effectiveness when a CO_2 source is present has not been tested yet. The goal of the present study was to find an affordable and effective active bed bug monitor. The objectives of this study were—1) to determine if interceptors, sugar–yeast monitors, and dry ice monitors are equally effective for trapping bed bugs, and 2) to determine if adding an experimental chemical lure can significantly increase the effectiveness of a sugar–yeast monitor.

Materials and Methods

Measurement of CO_2 Release Rates in Laboratory. CO_2 release rates from a sugar–yeast mixture and dry ice were first measured under laboratory conditions at 25°C. Based on literature and our preliminary experiments, a sugar, yeast, and water formulation and a quantity of dry ice that can produce an average of 400 ml/min CO_2 for 8 h was used.

Sugar–Yeast. A 19-liter plastic container was filled with a mixture of 150 g Lesaffre baker's yeast (Lesaffre Yeast Corporation, Milwaukee, WI), 750 g granulated cane sugar (U.S. Sugar Co. Inc., Buffalo, NY), and 3 liter warm water (40°C). All the ingredients were mixed for 3 min, and the container was sealed completely leaving a 5-mm-diameter outlet for measuring the CO_2 release rate. The CO_2 release rate was determined as milliliters of bubble fluid displaced by CO_2 per unit of time using a Bubble-O-Meter (Bubble-O-Meter, Dublin, OH). The CO_2 release rates were recorded hourly for 8 h from three containers.

Dry Ice. A 1.2-liter insulated jug (Coleman Company Inc., Wichita, KS) containing 400 g dry ice pellets was used. The flip-top spout of the jug was opened slightly allowing CO_2 to escape into the atmosphere. Weight loss of dry ice was recorded hourly for 8 h from three jugs. The CO_2 release rate was determined as amount of weight loss of dry ice over time. Volume of CO_2 released from dry ice at room temperature was calculated using the formula: $V = \text{weight loss (g)} / 44\,\text{g}\,CO_2 \times RT/P$, where $R = 0.0821$ liter atm $mol^{-1} K^{-1}$, $T = 298$ K, $P = 1$ atm.

Field Comparison Among the Sugar–Yeast Monitor, Dry Ice Monitor, and Climbup Insect Interceptors for Detecting Bed Bugs.

Study Site and Selection of Apartments. This study was conducted in two high-rise apartment buildings located at Irvington, NJ. Most of the apartments were occupied by one or two adult persons. To select test apartments, 6–18 interceptors were placed under bed and sofa legs (or beside the furniture legs if the legs were too large) in apartments with previous infestation history or current infestation. If the furniture did not have legs, the interceptors were placed beside the corners of the furniture. The interceptors were examined after 11-d. Most of the bed bugs captured in the interceptors were dead; therefore, all the bed bugs in the interceptors were discarded. Thirteen apartments (three one-bedroom and 10 studio apartments) with 22–516 bed bugs based on interceptors (6–18) were selected.

Sugar–Yeast Monitor. This monitor consisted of a 19-liter plastic bucket (Purina; Target, Minneapolis, MN) and two pitfall traps. The bucket was filled with a mixture of yeast, sugar, and water as described in the laboratory experiment (Fig. 1a). All the ingredients were added and mixed thoroughly immediately before installation. An inverted plastic dog bowl (600 ml volume, 18 cm diameter, 6.4 cm depth, and 1 mm thickness; IKEA, Baltimore, MD; Fig. 1a) was used as a pitfall trap. The outer wall of the dog bowl 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). The inside surfaces were coated with a light layer of talcum powder to make the traps slippery and prevent trapped bed bugs from escaping. The plastic bucket holding the fermenting materials was placed on top of two pitfall traps for catching bed bugs that were attracted to the bucket containing the sugar–yeast mixture. A 0.7-ml plastic centrifuge tube containing 400 µl of an experimental chemical lure dispensed onto cotton was placed in one of two pitfall traps placed under the bucket. The lid of each tube was left open to release the chemical vapor into the air. The experimental chemical lure consisted of nonanal, L-lactic acid (Sigma-Aldrich Co., St. Louis, MO), 1-octen-3-ol, and spearmint oil (Bedoukian Research Inc., Danbury, CT; 1:1:1:0.5 ratio). The lure used was slightly different than that reported (nonanal, 1-octen-3-ol, spearmint oil, and coriander Egyptian oil) by Singh et al. (2013); however, it was found at least equally effective as that described by Singh et al. (2013) in our preliminary laboratory assays.

Dry Ice Monitor. The dry ice monitor consisted of an insulated jug containing 400 g dry ice pellets as described in the laboratory experiment and two pitfall traps. The lid of the jug was opened slightly allowing CO_2 to escape into the atmosphere (Fig. 1b). Two pitfall traps were deployed 24 cm apart in a similar fashion as the sugar–yeast monitor. One trap received a dry ice jug and an experimental chemical lure, and the other one was nonbaited (Fig. 1b).This set up allowed for a fair comparison between the dry ice monitor and the sugar–yeast monitor.

Immediately after taking the counts from interceptors in the 13 apartments, one monitor was deployed in each apartment. Seven sugar–yeast and six dry ice monitors were deployed on the first night. On the

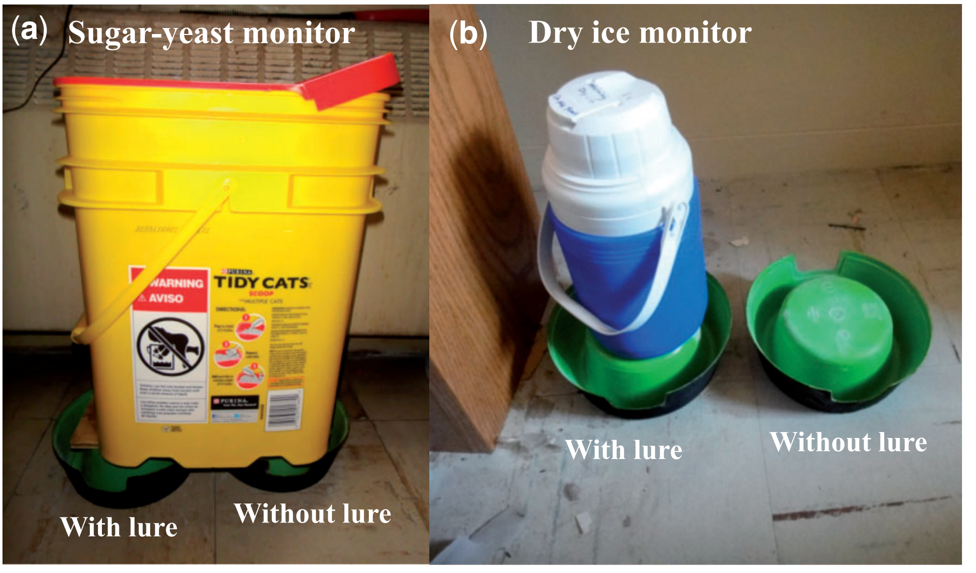

Fig. 1. Field set up for determining the efficacy of: (a) Sugar–yeast monitor or an experimental chemical lure, and (b) Dry ice monitor.

second night, the type of monitor in each apartment was switched, providing 13 replicates for each monitor. Monitors were only placed near sleeping areas of the residents where bed bugs were likely to be present. The numbers of bed bugs caught in the pitfall traps were recorded after 24 h. The dry ice monitors released CO_2 continuously for about 10 h after set up (between 6–7 p.m.), whereas the sugar–yeast monitor continued to release CO_2 until the monitors were taken down (approximately 24 h). However, the CO_2 release rates declined to less than 20 ml/min after 10 h in both the monitors based on laboratory observations.

Effectiveness of an Experimental Chemical Lure for Attracting Bed Bugs. The same sugar–yeast monitors used in the previous experiment were used for evaluating the attractiveness of the experimental chemical lure. Same as the previous experiment, a chemical lure-baited and a nonbaited pitfall trap were placed under each bucket. The difference from the previous experiment was that we recorded the bed bug counts from each of the two pitfall traps under each bucket separately. One or two sugar–yeast monitors were placed near the sleeping or resting areas in each test apartment. When two monitors were placed in an apartment, the monitors were in two different rooms (bedroom and living room). A total of nine monitors were placed in three one-bedroom and three studio apartments for one night. The numbers of bed bugs caught in the pitfall traps with or without lure were recorded next day.

Statistical Analyses. The CO_2 release rates at different observation periods were analyzed using Mixed model (JMP 2014) to determine the effect of CO_2 source (sugar–yeast and dry ice), time, and their interaction. For field experiments, the bed bug numbers captured in interceptors or monitors were $\log_{10}$ transformed to meet assumptions of normality and homogeneity of variance (Zar 1999). The initial bed bug count in interceptors was analyzed by one-way analysis of variance (ANOVA) to determine if there are any differences among the apartments randomly assigned to sugar–yeast and dry ice monitors. ANOVA was conducted to compare the bed bug counts among interceptors, sugar–yeast monitors, and dry ice monitors. Means were separated using Tukey's HSD test. A paired t-test ($P = 0.05$) was used to compare the bed bug counts in pitfall traps with lure and those without lure. All analyses were conducted using JMP version 11 (SAS Institute 2012).

Results and Discussion

CO_2 Release Rates. An average (± SEM) of 405.1 ± 45.5 and 397.0 ± 23.1 ml/min CO_2 was released by sugar–yeast and dry ice, respectively, during 8 h after activation (Fig. 2). The release rates were not significantly different between the two CO_2 sources ($F = 1.1$; df = 1, 16; $P = 0.32$). It should be noted that the dry ice completely sublimated after approximately 9–10 h, whereas the sugar–yeast continued to release CO_2 at lower rates after 8 h. The sugar–yeast container was much bulkier than the dry ice container for producing the similar release rates.

Field Comparison Among the Sugar–Yeast Monitor, Dry Ice Monitor, and Climbup Insect Interceptors for Detecting Bed Bugs. There were no significant differences in the initial mean bed bug counts among the apartments assigned to sugar–yeast and dry ice monitors ($F = 0.1$; df = 1, 11; $P = 0.92$). Interceptors (11-d placement of 6–18 interceptors), sugar–yeast monitors (1-d placement), and dry ice monitors (1-d placement) caught an average (± SEM) of 153.0 ± 42.6, 109.0 ± 30.1, and 85.5 ± 24.4 bed bugs, respectively (Fig. 3). There were no significant

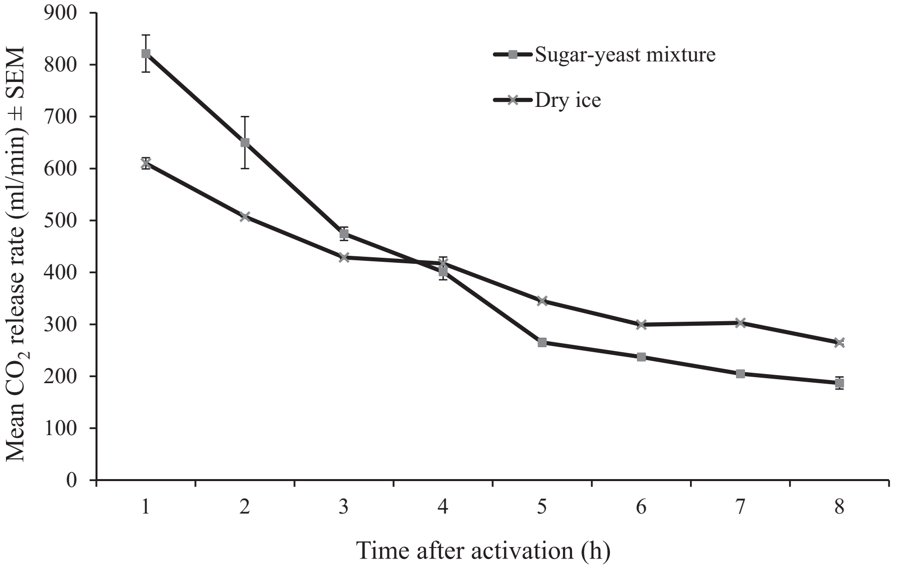

Fig. 2. CO_2 release rates from sugar–yeast–water mixture or dry ice. Warm water (40°C) was added to sugar–yeast and stirred for 3 min. The room temperature was 25°C.

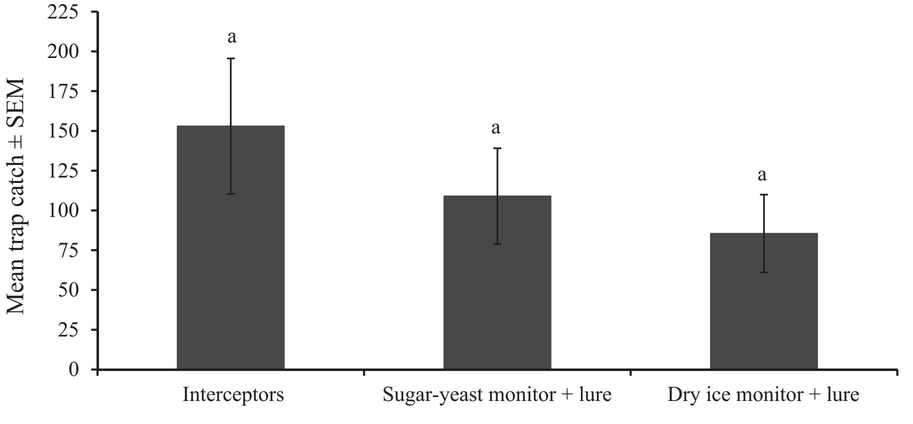

Fig. 3. Field comparison among the interceptors (11-d placement of 6–18 interceptors per apartment), sugar–yeast monitor (1-d placement of one monitor per apartment), and dry ice monitor (1-d placement of one monitor per apartment) for detecting bed bugs in occupied apartments. The sugar–yeast–water mixture was 150 g yeast, 750 g granulated cane sugar, and 3 liter warm (40°C) water. The dry ice monitor contained 400 g dry ice pellets. An experimental chemical lure was added to sugar–yeast and dry ice monitor. Bars with same letters are not significantly different ($P > 0.05$, Tukey's HSD test). Analysis was based upon logarithmic transformed data, but actual mean values are shown.

differences in trap catch among the three monitors ($F = 2.0$; df = 2, 36; $P = 0.14$).

The results indicate that the sugar–yeast monitor is equally effective as the dry ice monitor in trapping bed bugs. A previous study by Singh et al. (2013) reported no significant difference in trap catch between traps baited with sugar–yeast and traps baited with CO_2 derived from gas cylinders in low-level infested apartments. The current study confirms that sugar–yeast fermentation can be used as an alternative CO_2-generating method for bed bug monitors.

Wang et al. (2011) found 1-d deployment of a dry ice monitor was equally effective as 7-d trapping with interceptors placed under furniture (sofa and bed) legs. Similarly, our results show 1-d deployment of a dry ice or sugar–yeast monitor is equally effective as 11-d placement of interceptors under furniture legs. The current study is different from the Wang et al. (2011) study in that less dry ice was used in this study (400 vs. 1100 g). Higher CO_2 release rate and greater detection efficacy can be achieved by increasing the quantity of the sugar–yeast materials; however, the container size will need to be larger and become very cumbersome. To increase the probability of detecting very low infestations, the sugar–yeast monitor can be re-filled daily for multiple days. Sugar–yeast monitor provides faster results than using interceptors, and this advantage is most obvious for monitoring bed bugs in sensitive environments where fast results are needed. It provides a simple and affordable do-it-yourself option for consumers.

Effectiveness of an Experimental Chemical Lure for Attracting Bed Bugs. The mean ($\pm$SEM) bed bug count in interceptors in six bed bug-infested apartments during 11-d placement was 284.6 ± 80.0. Pitfall traps baited with a chemical lure caught an average of 183.1 ± 66.4 bed bugs compared with 25.3 ± 8.2 bed bugs trapped in those without lure during one night trapping period. The differences between the bed bug counts were highly significant ($t = -5.3$, $df = 8$, $P = 0.0008$). Previous studies showed traps baited with a chemical lure (nonanal, 1-octen-3-ol, spearmint oil, and coriander Egyptian oil) caught 2.5 and 2.2 times more bugs than their corresponding nonbaited controls in a laboratory (Singh et al. 2012) and field study (Singh et al. 2013), respectively. Since then, we modified the lure formula by replacing coriander Egyptian oil with L-lactic acid. This field study showed a much greater difference in trap catch (7.2 times) when the chemical lure was used with a sugar–yeast monitor (CO_2 source) compared with the chemical lure alone described in Singh et al. (2012, 2013). The results suggest that the chemical lure is very effective in improving the trap catch for monitors that use CO_2 as a long-range attractant.

This sugar–yeast monitor with an attractive bed bug lure delivers an affordable and effective solution for monitoring bed bugs. This monitor is more convenient than those using dry ice or CO_2 cylinder, as all materials are readily available. Our study was conducted in occupied apartments where the monitor had a direct competition with the human host in attracting bed bugs. The sugar–yeast monitor would be expected to be more effective in vacant rooms and nontraditional locations such as schools, hospitals, offices, and theaters etc. where a host is temporarily not present. Some general safety precautions must be followed while using the sugar–yeast monitor: 1) The monitor should be properly secured, 2) The lid should be properly placed on the sugar–yeast container, 3) The monitor should be kept away from children or pets, and 4) The sugar–yeast solution should be disposed of immediately after use. This monitor is more affordable compared with existing active monitors. There is initial cost of approximately US$10 for buying a plastic bucket and two pitfall traps and then the operating cost (sugar and yeast) is only US$1.7 per night. Disadvantage of the sugar–yeast monitor is that a large container is needed for holding sufficient sugar, yeast, and water. These monitors also need to be washed after use. In spite of these disadvantages, this monitor appears to be a promising active monitor for monitoring bed bugs.

Future studies should include modifying the design of the sugar–yeast container to minimize the size needed for generating sufficient CO_2 and make it more appealing to consumers. The volatile organic compounds produced by yeast which are similar to that found in human emanations (Hazelwood et al. 2008; Smallegange et al. 2005, 2009) need to be studied for their attractiveness to bed bugs.

Acknowledgments

We thank the management and staff of a private property management group in Irvington, New Jersey, for allowing us to conduct field experiments in their facility. This project was funded by a grant from the U.S. Department of Urban and Housing Development Healthy Homes Technical Studies grant program. Partial support was provided by the New Jersey Agricultural Experiment Station and by the U.S. Department of Agriculture–National Institute for Food and Agriculture, Hatch Multistate project number NJ08127. This is New Jersey Experiment Station publication D-08-08117-10-14.

References Cited

Dekkar, T., and W. Takken. 1998. Differential responses of mosquito sibling species *Anopheles arabiens*is and *An. Quadriannulatus* to carbon dioxide, a man or a calf. Med. Vet. Entomol. 12: 136–140.

Hazelwood, L. A., J.-M. Daran, A. J. A. van Maris, J. T. Pronk, and J. R. Dickinson. 2008. The Ehrlich pathway for fusel alcohol production: A century of research on *Saccharomyces cerevisiae* metabolism. Appl. Environ. Microbiol. 74: 2259–2266.

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, R. C. Smallegange, W. Takken, and D. J. Conway. 2011. Field testing of different chemical combinations as odour baits for trapping wild mosquitos in the Gambia. PLoS ONE 6: e(1967)6.

JMP 2014. Repeated Measures Analysis (Mixed Model). (http://www.jmp.com/academic/pdf/learning/07_repeated_measures_analysis_mixed_model.pdf) (accessed 2 July 2014).

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 application, pp. 52–54. W. B. Saunders Co., Philadelphia, PA.

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.

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.

Oli, K., J. Jeffery, and I. Vythilingam. 2005. A comparative study of adult mosquito trapping using dry ice and yeast generated carbon dioxide. Trop. Biomed. 22: 249–251.

Russell, R. C. 2004. The relative attractiveness of carbon dioxide and octenol in CDC-and EVS-type light trap for sampling the mosquitoes *Aedes aegypti* (L.), *Aedes polynesiensis* Marks and *Culex quinquefasciatus* Say in Moorea, French Polynesia. J. Vector Ecol. 29: 309–314.

Saitoh, Y., J. Hattori, S. Chinone, N. Nihei, Y. Tsuda, H. Kurahashi, and M. Kobayashi. 2004. Yeast-generated CO_2 as a convenient source of carbon dioxide for adult mosquito sampling. J. Am. Mosq. Control Assoc. 20: 261–264.

SAS Institute. 2012. JMP statistics and graphics guide, version 11 of JMP. SAS Institute, Cary, NC.

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: 273613. doi:10.1155/2012/273613.

Singh, N., C. Wang, and R. Cooper. 2013. Effect of trap design, chemical lure, carbon dioxide release rate, and source of carbon dioxide on efficacy of bed bug monitors. J. Econ. Entomol. 106: 1802–1811.

Smallegange, R. C., Y. T. Qiu, J. J. A. van Loon, and W. Takken. 2005. Synergism between ammonia, lactic acid and carboxylic acids as kairomones in the host seeking behaviour of the malaria mosquito *Anopheles gambiae* sensu stricto (Diptera: Culicidae). Chem. Senses 30: 145–152.

Smallegange, R. C., Y. T. Qiu, G. Bukovinszkine-Kiss, J. J. A. van Loon, and W. Takken. 2009. The effect of aliphatic carboxylic acids on olfaction-based host-seeking of the malaria mosquito *Anopheles gambiae* sensu stricto. J. Chem. Ecol. 35: 933–943.

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.

Wang, C., T. Gibb, and G. W. Bennett. 2009. Evaluation of two least toxic integrated pest management programs for managing bed bugs (Heteroptera: Cimicidae) with discussion of a bed bug intercepting device. J. Med. Entomol. 46: 566–571.

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.

Xue, R. D., M. A. Doyle, and D. L. Kline. 2008. Field evaluation of CDC and Mosquito Magnet X traps baited with dry ice, CO_2 sachet, and octenol against mosquitoes. J. Am. Mosq. Control Assoc. 24: 249–252.

Zar, J. H. 1999. Biostatistical analysis, 4th edn. Prentice Hall, Upper Saddle River, NJ.

Received 21 September 2014; accepted 26 February 2015.