Field Evaluation of a Novel Trap Baited with Carbon Dioxide Produced by Yeast for the Collection of Female *Aedes aegypti* Mosquitoes in Mexico

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Abstract. A trap made from low-cost materials and using an attractant of a yeast mixture producing carbon dioxide was designed and evaluated to collect adult *Aedes aegypti* (L.) mosquitoes. The Trap Mosquito Box prototype was tested against the "standards" BG-Sentinel traps and CDC backpack aspirator in the field. The mean numbers of mosquitoes (± standard deviation) caught by the three different collection methods were: Trap Mosquito Box 2.42 (± 3.08), BG-Sentinel trap 2.86 (± 3.71), and backpack aspirator 0.59 (± 0.90). Statistical tests showed the Trap Mosquito Box and BG-Sentinel trap were equally effective in collecting *A. aegypti* and both methods were significantly different than the backpack aspirator. Emission of carbon dioxide produced by the yeast mixture was greatest during the first hours after incubation in a laboratory and captured the most mosquitoes in the Trap Mosquito Box. Production of carbon dioxide [$Y = -631.24 + 941.26 (\log x)$] and the rate of mosquitoes captured per time period [$Y = 20.29 + 23.50 (\log x)$] were best explained by logarithmic regressions. Advantages and disadvantages of the Trap Mosquito Box for mosquito surveillance are discussed.

Introduction

Dengue is the most important vector-borne arbovirosis transmitted by *Aedes aegypti* (L.) mosquitoes. Because vector control is the only feasible strategy to fight transmission of dengue, it is of paramount importance to obtain reliable data by monitoring. A variety of surveillance schemes have been developed, including the use of ovitraps (Reiter et al. 1991) and larval or *Stegomyia* indices (e.g., House index, Breteau index, or Container index) (PAHO 1994). However, the *Stegomyia* indices are limited in assessing risk of transmission (Focks 2003). Another approach launched a few years ago used pupal demographic indices to assess vector abundance in relation to human population (Strickman and Kittayapong 2003, Focks and Alexander 2006). Several countries joined this initiative and evaluated the usefulness of pupal demographic indices, including recommendations on the best ways to collect and count pupae from containers (Romero-Vivas et al. 2007). Although pupal indices can be used to closely estimate vector abundance in an area, they are time-consuming and require well-trained personnel and large sample sizes to obtain robust and accurate data. Collecting and counting directly the number of female *A. aegypti* mosquitoes attracted to humans in endemic areas might be used to best estimate vector abundance, thereby providing a way to assess risk of transmission of dengue. However, use of host-seeking female *A. aegypti* collected from human volunteers is ethically questionable (Kröckel et al. 2006) because of the risk of infection. To overcome this situation, several methods are available to catch adult mosquitoes, although they vary in their effectiveness to capture female *A. aegypti*. Two methods to collect adults that can be considered as the “standards” to estimate vector abundance are BG-Sentinel™ traps (Biogents AG, Regensburg, Germany) (Kröckel et al. 2006) and the CDC backpack aspirator (model 1412, John W. Hock Company, Gainesville, FL) (Clark et al. 1994). A common denominator between these two methods is the expense associated with their purchase, maintenance, and operation.

It is well known that mosquitoes (including *A. aegypti*) are attracted to carbon dioxide (Gillies 1980). Many field studies with other mosquito species reported the collection of hungry females by using a combination of light traps supplemented with pellets of dry CO_2 (e.g., Chen et al. 2010). Commercially available dry ice has two shortcomings: it is expensive in the long-term or in large-sized studies, and it is virtually impossible to obtain in some places such as remote areas of developing countries. Yeast utilizes different carbohydrate substrates to produce either carbon dioxide or ethanol as secondary by-products. Carbon dioxide produced by yeast has been reported as an effective attractant to collect nymphal stages of triatomine bugs (Guerenstein et al. 1995, Pires et al. 2000, Pimenta et al. 2007) and mosquitoes (Saitoh et al. 2004, Smallegange et al. 2010). The main objective of this study was to design a trap prototype to compare with the standards for collecting adult mosquitoes under semi-field and field conditions.

Materials and Methods

A laboratory strain of *A. aegypti* maintained at the insectary facilities of Laboratorio de Entomología Médica, Universidad Autónoma de Nuevo León (FCB-UANL) was used. Standard protocols (Pérez et al. 2004) were used to rear the mosquitoes.

An attractant yeast mixture was prepared by mixing 200 ml of water, 50 g of sugar, and 1 g of baker´s dry yeast *Saccharomyces cerevisiae* Meyen *ex* E.C. Hansen (Levadura Azteca™, S. A. de C.V.DF, México). A system of carbon dioxide production using two plastic bottles was prepared following the same proportions described by Saitoh et al. (2004): bottle A = 750 ml water, 75 g sugar, and 6 g dry yeast, and bottle B = 850 ml water, 50 g sugar, and 3 g dry yeast. Preparation of the attractant mixture was initiated by completely dissolving sugar powder in water, after which dry yeast was carefully added to achieve a homogenous mixture.

The Trap Mosquito Box was created from a 12 x 14 x 22 cm black plastic box normally used to store index cards. The box was positioned so one of its sides became the bottom. A circular portion was removed from the top and a plastic screw lid glued onto the edges of the hole. This screw lid acted as a fixing mechanism for the mosquito collection bag manufactured using a piece of black fine-mesh fabric. A square-framed electric fan (Electrónica Steren™ S.A. de C.V, DF, México) 10 cm in diameter and operated at 117 volts AC and 2,500-3,000 rpm was fixed in the middle inside the trap. The electrical fan was used to help disperse carbon dioxide outside of the trap and to farther distances. A plastic sandwich-type container with an air-tight lid was put onto the bottom of the trap. The lid was modified by removing a square, leaving a hole that was covered by plastic mesh to allow carbon dioxide to diffuse upward. At 2.5 cm above the bottom of the container and on each of the four sides of the box, a rectangular portion of the wall was removed to create dispersion windows covered with fine-mesh fabric (Fig. 1).

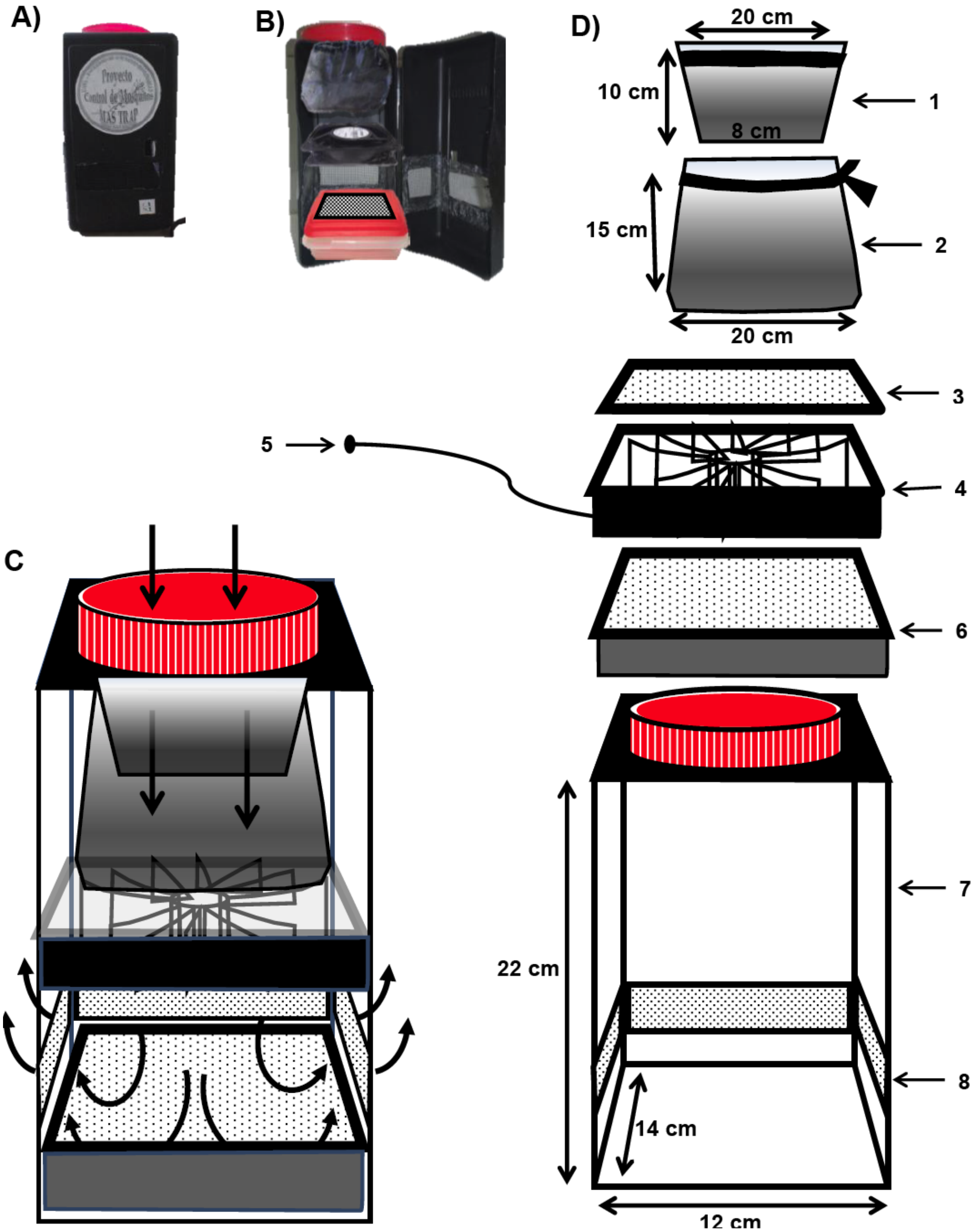


Fig. 1. Trap Mosquito Box prototype closed box (A), open box showing the inside (B), schematic depiction of direction of air flow (C), and all components of the Trap Mosquito Box (D): 1. plastic funnel, 2. collecting cloth bag, 3. top mesh screen, 4. electrical fan, 5. cable, 6. bottom mesh screen, 7. plastic wall of the box, and 8. lateral mesh screen. Dotted red lines represent the air flow of carbon dioxide and solid blue lines represent the direction of entrance of mosquitoes into the trap.

Production of CO_2 and rate of mosquitoes captured by the different traps were analyzed in a laboratory. For logistical reasons and limited space in the insectary facilities, carbon dioxide production and capture rate were evaluated separately, although both assays used the same methodology and experimental conditions. Production of carbon dioxide was measured using a volumetric respirometer (Carmona et al. 2004) that allowed quantification of the CO_2 volume produced per unit of time. The volume in milliliters of carbon dioxide produced by the attractant yeast mixture was recorded at 1, 2, 4, 8, 16, and 32 hours.

The capture rate of mosquitoes was assessed by using the same attractant yeast mixture in the Trap Mosquito Box prototype. In the insectary facilities, 100 unfed 3-day-old female *A. aegypti* were released into each of two experimental cages of 2 m^3. After allowing a few minutes for acclimatization of the mosquitoes, the Trap Mosquito Box prototype containing the attractant yeast mixture was introduced. Taking care not to disturb the experimental mosquitoes, the mosquito collection bag was replaced at the time periods described previously. Collection bags were placed onto a chill-table to allow counting of mosquitoes caught at each interval of time. If all the mosquitoes had been captured before 32 hours, the Trap Mosquito Box prototype was withdrawn. Bioassays to measure carbon dioxide production and determine the capture rate were each replicated 10 times, with a new batch of mosquitoes (n = 100) used for each replication.

A field experiment was used to compare the capture effectiveness of the Trap Mosquito Box with two methods considered as standards. Based on information of dengue cases for 2011, an area in the municipality of Escobedo, N.L. was selected. Although the study was to be done during the rainy season, rain was sparse during the actual period selected. Environmental parameters (mean temperature of 32 ± 2°C and mean relative humidity of 27 ± 3%) were recorded daily. In the study area, three houses per block were selected randomly and in each home one of the three traps was evaluated. Sampling with a backpack aspirator was done between 1300 and 1500 hours, while Trap Mosquito Box and BG-Sentinel traps were deployed at approximately the same time and left operating for 24 hours. The Trap Mosquito Box and BG-Sentinel were placed outside of houses, and backpack aspirations were done for a standardized period of 20 minutes inside the houses. Mosquitoes caught by the aspirator were maintained in collection plastic cups and transported in an ice chest to the laboratory. Mosquitoes collected the following day in the Trap Mosquito Box or BG-Sentinel were handled in the same way. Once in the laboratory, mosquitoes caught by any of the methods were placed onto a chill-table and sorted with the aid of a dissecting microscope into species and sex. Only data on female *A. aegypti* are reported in this article. Originally, the experiment was scheduled to have 30 replications per method, but the final sample size for each method was variable because of unforeseen circumstances.

For each set of results, the criteria for the normality of data were tested by using Anderson-Darling´s test (Minitab™ v. 11.0, Minitab Inc., Coventry, UK). In some cases, data were transformed (x + 1) to normalize distribution, and when the assumption could not be achieved, a non-parametric test was used to analyze data. Quantification of carbon dioxide production and rate of captured female mosquitoes in the Trap Mosquito Box were estimated by regression analysis, considering the mean number of mosquitoes caught as the dependent variable (Y) and time period as the independent variable (X). Significance of regression analysis was determined by ANOVA. All statistical tests were considered significant if $p < 0.05$.

Results

The volume of CO_2 produced by the yeast mixture was not linearly related to the time periods evaluated. After incubation of the microbial mixture, the metabolic production of carbon dioxide by yeasts was greatest during the first hours and later steadily decreased until 32 hours after incubation. The relationship between the amount of carbon dioxide produced by time periods was best explained by a logarithmic regression [Y = -631.24 + 941.26 (log x)] (Fig. 2A), which was statistically significant ($F = 13.55$; df = 1, 4; $p < 0.05$).

The rate of mosquitoes captured by the Trap Mosquito Box prototype was greatest after the first hours of exposure to the microbial attractant mixture. Female *A. aegypti* mosquitoes were caught in the Trap Mosquito Box mostly within the first time periods. On a few occasions, 100% of mosquitoes were captured even before reaching the last time period of 32 hours after incubation. Similarly, in the results obtained on the rate of production of carbon dioxide by yeast, during the evaluation of the Trap Mosquito Box prototype, the response of mosquitoes to the attractant bait was a non-linear relationship. The model that described this relationship was [Y = 20.29 + 23.50 (log x)] (Fig. 2B), which was very significant ($F = 102.82$; df = 1, 4; $p < 0.01$).

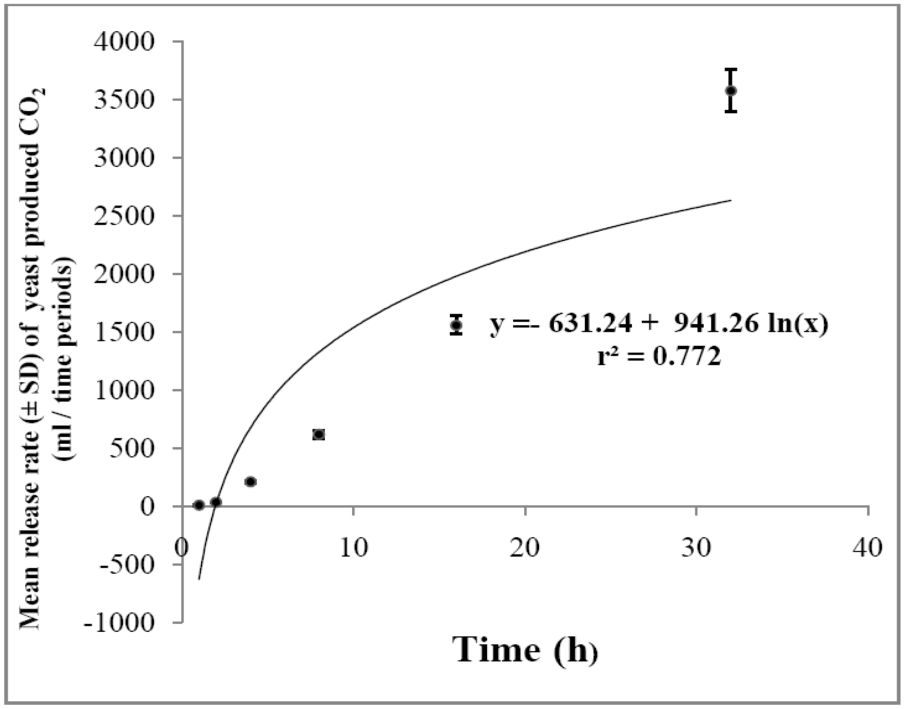
The number of mosquitoes captured was significantly different ($F = 22.52$; df = 2, 80; $p < 0.01$) for the three traps. Results from Tukey´s test (q = 3.37; df = 3,80; $p < 0.05$) showed the mean numbers of mosquitoes collected with the Trap Mosquito Box and BG-Sentinel were not statistically different from each other and that these two methods had greater means than that obtained by the backpack aspirator (Table 1).

Discussion

This was the first evaluation of an adult trap prototype baited with yeast-generated carbon dioxide for the collection of *A. aegypti* vectors in Mexico. These data demonstrated that yeast activity was most intense during the first hours of exposure, and also showed that production of CO_2 continued up to 32 hours. Mosquitoes seemed to be more attracted to the trap when the production of carbon dioxide by yeast was greatest. Saitoh et al. (2004) also showed that carbon dioxide production was more intense during the first hours after incubation of yeast and continued to be released up to 30 hours. Quantification of carbon dioxide produced by the yeast mixture agreed well with the results of Saitoh et al. (2004), and our contribution demonstrated that the period of greatest release of CO_2 followed a similar pattern as the rate of mosquitoes captured in the Trap Mosquito Box. In addition to the production of carbon dioxide by yeast, Smallegange et al. (2010) suggested that during the microbial activity other volatile compounds released might be attractive to mosquitoes. In the present study, no efforts were made to detect or quantify volatiles in the attractant yeast mixture. In terms of the applicability of the findings of this study, we propose the Trap Mosquito Box can be used in endemic areas for a maximum period of 24 hours because most of the mosquitoes would have been caught by then.

Field evaluation of the Trap Mosquito Box prototype against the other two standard methods showed the Trap Mosquito Box was as effective as the BG-Sentinel and captured more mosquitoes than the backpack aspirator. The number of mosquitoes caught by backpack aspirator during a 15-minute period may not be

A



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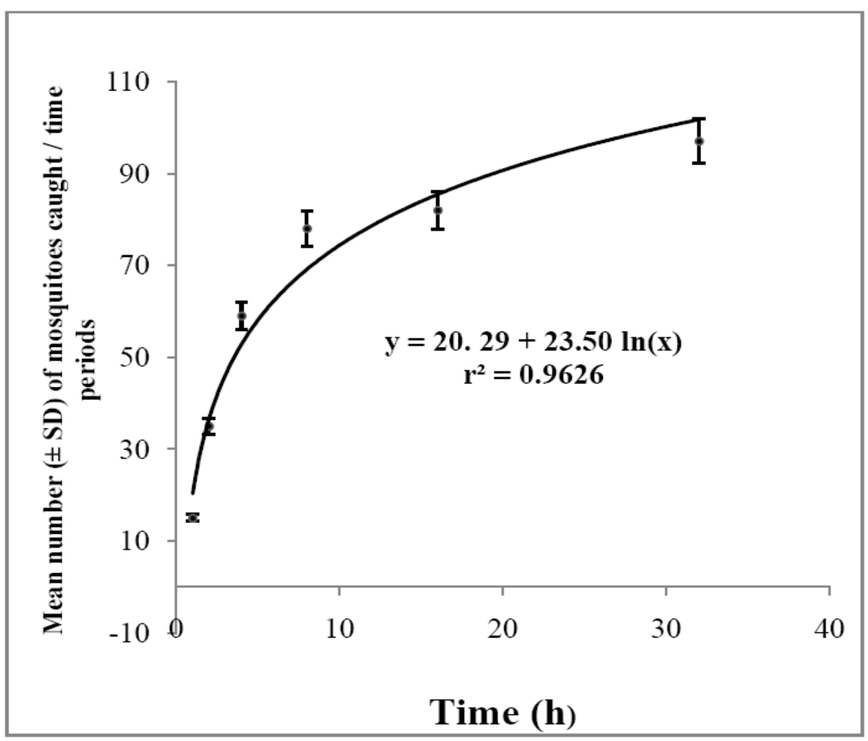

Fig. 2. Plot A represents the rate of mean (± SD) carbon dioxide production by the yeast mixture in relation to different time periods (1, 2, 4, 8, 16, and 32 hours) after incubation. Plot B represents the mean rate (± SD) of the number of female *A. aegypti* mosquitoes caught at the different time intervals. Regression equations are written in each plot.

Table 1. Total Number of Mosquitoes Caught per House Premises During the Field Evaluation of the Trap Mosquito Box Prototype Compared with Two Standards for Sampling Adult *A. aegypti* Mosquitoes

Replication	Trap Mosquito Box	BG Sentinel	Backpack aspirator
1	0	0	2
2	7	1	2
3	1	7	2
4	3	2	2
5	1	10	0
6	5	7	1
7	0	1	0
8	3	2	0
9	0	0	0
10	1	1	1
11	0	0	0
12	1	2	0
13	0	2	3
14	0	4	0
15	2	6	0
16	3	0	0
17	2	3	0
18	12	8	0
19	0	0	0
20	8	3	0
21	5	2	0
22	4	0	1
23	0	0	0
24	0	0	0
25	-	15	0
26	-	7	2
27	-	0	1
28	-	0	0
29	-	0	0
30	-	-	0
n	58	83	17
Mean	2.42	2.86	0.59
SD	3.08	3.71	0.90
Range	(0-12)	(0-15)	(0-3)

directly comparable with the number of mosquitoes caught by the Trap Mosquito Box or BG-Sentinel that were left in the place for 24 hours. Notwithstanding, backpack aspirations were carried out as they would have been under normal or routine field sampling and in other studies (Williams et al. 2006). BG-Sentinel and backpack aspirators were equally compared. It is important to highlight the fact that the observed effectiveness of the Trap Mosquito Box design was equivalent to that achieved by the "standard" BG-Sentinel. Schoeler et al. (2004) reported CDC backpack aspirators equally effective as collections of female *A. aegypti* landing on humans. In contrast, in studies in Australia (Williams et al. 2006) and Brazil (Maciel-de-Freitas et al. 2006), the number of female *A. aegypti* mosquitoes caught by BG-Sentinel devices was greater than that obtained by backpack aspirators. Results from the field experiment comparing the Trap Mosquito Box and the other two standard methods were similar to the previously-mentioned articles reporting BG-Sentinel to be more effective than the backpack aspirator. Williams et al. (2006) found that blood-fed females were more frequently collected by backpack aspirators, whereas unfed nulliparous females were more attracted to BG-sentinel traps, a finding also reported by Maciel-de-Freitas et al. (2006) who found that more female *A. aegypti* were captured at the initial stages of ovarian development. In the present study, neither the trophic nor ovarian stages were recorded; although it was hypothesized that because the Trap Mosquito Box utilized carbon dioxide as an attractant, it was expected that mosquitoes trapped were unfed females at early stages of ovarian development.

What are the advantages and disadvantages of the Trap Mosquito Box prototype? The low cost of the trap ($20 USD) is an advantage over the other commercial traps that cost $200-720 USD. Furthermore, the components to build a trap can be found in any general hardware or stationery store, and the assemblage of parts can be easily made. For vector control and surveillance programs in developing countries, the large-scale use of low-cost traps is an urgent necessity. A disadvantage of the trap might be its need to be plugged into an AC outlet. Residents in some houses did not like having an electrical appliance turned on for 24 hours. Acceptance of the trap could be improved by involving social medical workers or anthropologists. Another alternative is to modify the Trap Mosquito Box prototype to use an external DC energy source, although this might increase its cost. Finally, developing countries should promote and invest resources to study and search for alternative low-cost technologies which in turn might lead to discovery of new and feasible sampling and control strategies.

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References Cited

Carmona, J. F., J. F. Vázquez, A. Martín, and A. Chica. 2004. Diseño y construcción de un respirómetro. *In* M. A. Mañanas [eds.], Bioingenieria. Proceedings of the XXV Jornadas de Automática. Ciudad Real. Universidad de Castilla la Mancha, 8-10 Septiembre.

Chen, Y. C., C. Y. Wang, H. J. Teng, C. F. Chen, M. C. Chang, L. C. Lu, C. Lin, S. W. Jian, and H. S. Wu. 2010. Comparison of the efficacy of CO_2-baited and unbaited light traps, gravid traps, backpack aspirators, and sweep net collections for sampling mosquitoes infected with Japanese encephalitis virus. J. Vector Ecol. 36: 69-74.

Clark, G. G., H. Seda, and D. J. Gubbler. 1994. Use of the “CDC backpack aspirator” for surveillance of *Aedes aegypti* in San Juan, Puerto Rico. J. Amer. Mosq. Control Assoc. 10: 119-124.

Focks, D. 2003. A review of entomological sampling methods and indicators for dengue vectors. UNICEF/UNDP/WORLD BANK/WHO. Special Programme for Research and Training in Tropical Diseases (TDR) (TDR/IDE/DEN/03.1)

Focks, D., and N. Alexander. 2006. Multicountry study of *Aedes aegypti* pupal productivity survey methodology: findings and recommendations. UNICEF/ UNDP/WORLD BANK/WHO. Special Programme for Research and Training in Tropical Diseases (TDR). (TDR/IRM/DEN/06.1).

Gillies, M. T. 1980. The role of carbon dioxide in host-finding by mosquitoes (Diptera: Culicidae): a review. Bull. Entomol. Res. 70: 525-532.

Guerenstein, P. G., M. G. Lorenzo, J. A. Núñez, and C. R. Lazzari. 1995. Baker's yeast, an attractant for baiting traps for Chagas' disease vectors. Experientia 51: 834-837.

Kröckel, U., A. Rose, A. E. Eiras, and M. Geier. 2006. New tools for surveillance of adult yellow fever mosquitoes: comparison of trap catches with human landing rates in an urban environment. J. Amer. Mosq. Control Assoc. 22: 229-238.

Maciel-de-Freitas, R., A. E. Eiras, and R. Lourenço-de-Oliveira. 2006. Field evaluation of effectiveness of the BG-Sentinel, a new trap for capturing adult *Aedes aegypti* (Diptera: Culicidae). Mem. Inst. Oswaldo Cruz 101: 321-325.

Pan American Health Organization (PAHO). 1994. Dengue and dengue hemorrhagic fever in the Americas: guidelines for prevention and control. Scientific Publications 548. Washington, DC.

Pérez, O. R. J., J. A. Bisset, M. Leyva, M. Díaz, O. Fuentes, F. Ramos, R. González, and I. García. 2004. Manual de Indicaciones Técnicas para Insectarios, pp. 16-28. Ciencias Médicas, La Habana.

Pimenta, F. E., L. Diotaiuti, A. C. Lustosa-Lima, and M. G. Lorenzo. 2007. Evaluation of cultures of *Saccharomyces cerevisiae* as baits for *Triatoma dimidiata* and *Triatoma pallidipennis*. Mem. Inst. Oswaldo Cruz 102: 229-231.

Pires, H. H. R., C. R. Lazzari, L. Diotaiuti, and M. G. Lorenzo. 2000. Performance of yeast-baited traps with *Triatoma sordida, Triatoma brasiliensis, Triatoma pseudomaculata,* and *Panstrongylus megistus* in laboratory assays. Rev. Panam Salud Publica / Pan Amer. J. Public Health 7: 384-385.

Reiter, P., M. A. Amador, and N. Colon. 1991. Enhancement of the CDC ovitrap with hay infusions for daily monitoring of *Aedes aegypti* populations. J. Amer. Mosq. Control Assoc. 7: 52-55.

Romero-Vivas, C. M. E., H. Llinás, and A. K. I. Falconar. 2007. Three calibration factors, applied to a rapid sweeping method, can accurately estimate *Aedes aegypti* (Diptera: Culicidae) pupal numbers in large water-storage containers at all temperatures at which dengue virus transmission occurs. J. Med. Entomol. 44: 930-937.

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. Amer. Mosq. Control Assoc. 20: 261-264.

Schoeler, G. B., S. S. Scleich, S. M. Manweiler, and V. L. Sifuentes. 2004. Evaluation of surveillance devices for monitoring *Aedes aegypti* in an urban area of northeastern Peru. J. Amer. Mosq. Control Assoc. 20: 6-11.

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*. Malaria J. 9: 292.

Strickman, D., and P. Kittayapong. 2003. Dengue and its vectors in Thailand: calculated transmission risk from total pupal counts of *Aedes aegypti* and association of wing-length measurements with aspects of the larval habitat. Amer. J. Trop. Med. Hyg. 68: 209-217.

Williams, C. R., S. A. Long, R. C. Russell, and S. A. Ritchie. 2006. Field efficacy of the BG-Sentinel compared with CDC backpack aspirators and $C0_2$-baited EVS traps for collection of adult *Aedes aegypti* in Cairns, Queensland, Australia. J. Amer. Mosq. Control Assoc. 22: 296-300.