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Artículo Original

Laboratory and semi-field evaluation of inexpensive trap prototypes for the collection of Dengue vector mosquito *Aedes aegypti* (Diptera: Culicidae)

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RESUMEN

Evaluación en laboratorio y semi-campo de prototipos de trampas económicas para la colecta del vector del dengue, *Aedes aegypti* (Diptera: Culicidae)

Introducción. En la vigilancia entomológica del mosquito *Aedes aegypti*, vector del dengue, son utilizados una gran variedad de sistemas de trampeo. Sin embargo, existe la necesidad de disminuir el impacto económico de estos dispositivos en los programas de control de zonas endémicas, mediante el diseño de una trampa efectiva y de bajo costo.

Objetivo. Diseñar y evaluar diferentes prototipos de trampas para la colecta de mosquitos adultos *Aedes aegypti* mediante el uso de materiales de bajo costo y dióxido de carbono biológico producido por levaduras.

Materiales y Métodos. Cuatro prototipos de trampas fueron evaluados en condiciones de laboratorio y semi-campo. Los ensayos de laboratorio se realizaron en jaulas de 60 cm^3 donde se colocó un prototipo y se liberaron 25 hembras *Aedes aegypti* sin alimentación sanguínea. Posterior a un tiempo de exposición de 24 h, los prototipos fueron retirados y los mosquitos capturados fueron contabilizados. Para cada prototipo se realizaron cinco repeticiones y se utilizó un lote nuevo de mosquitos en cada repetición. En semi-campo, se realizó el mismo modelo experimental utilizando jaulas de 2 m^3 con un lote de 100 hembras y 15 repeticiones para cada prototipo. Todos los datos fueron analizados con una prueba de normalidad Anderson-Darling. El ensayo de laboratorio fue analizado con una prueba de varianza de una vía y una comparación múltiple de medias de Tukey. Los experimentos de semi-campo fueron analizados con las pruebas no paramétricas de Kruskal-Wallis y una comparación múltiple de medias por Nemenyi.

Resultados. En los ensayos realizados se observó una diferencia significativa entre los diseños evaluados. El mejor modelo fue el prototipo nombrado Trap Mosquito Box (TMB). El dispositivo capturó hasta 90% de la población de prueba a las 24 h del tiempo de exposición, tanto en laboratorio como en el ensayo de semi-campo.

Conclusiones. El modelo TMB representa una opción en la vigilancia y el control del mosquito *Ae. aegypti*. El prototipo puede reportar datos que ayuden en la predicción de riesgo de transmisión, facilitando las acciones preventivas en países con recursos económicos limitados.

Palabras clave: trampas adultos, dióxido de

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carbono, atrayente, ensayos de atracción, fermentación

ABSTRACT

Introduction. Entomological surveillance of dengue mosquito vector *Aedes aegypti* are usually carried out using any one of a variety of trapping systems. However many of the current systems are expensive and there is a need to devise new low-cost traps for the systematic use in control programs in endemic areas.

Objective. To design and evaluate different trap prototypes made of inexpensive materials baited with carbon dioxide produced by yeast for collecting *Aedes aegypti* adult mosquitoes

Materials and Methods. Four prototypes of low-cost traps were evaluated under laboratory and semi-field conditions. Laboratory tests were conducted in cages of 60 cm^3 where we placed individually the different prototypes and released 25 unfed *Aedes aegypti* females. After an exposure time of 24 h prototypes were removed from the cages and all mosquitoes captured were counted. For each prototype we included five repetitions using a new batch of mosquitoes in each repetition. In the semi-field test, we used the same experimental cage designs, scaled up to 2 m^3 cage volumes, using a batch of 100 females in each trial, replicated 15 times for each type of prototypical trap. All data were analyzed with using Anderson-Darling normality tests. The laboratory results were analyzed using parametric one-way ANOVA testing and Tukey´s test of multiple comparisons. Alternately, the semi-field experiments were analyzed a non-parametric Kruskal-Wallis test and multiple comparison of means was computed using Nemenyi´s test.

Results. From this two set of bioassays, we observed significant differences among the different trap designs. The best trap was a prototype named Trap Mosquito Box (TMB), where the model captured up to 90% of the test population within a period of 24 h of exposure time in laboratory and semi-field trials.

Conclusions. The model TMB represents a high-efficiency low-cost option for the surveillance and control of *Ae. aegypti*. TMB baited with carbon dioxide produced by yeast may help predicting the risk of transmission by providing good estimates of adult vector densities in a given area.

Key words: Adult traps, carbon dioxide, lure, attraction essay, fermentation

INTRODUCTION

The mosquito *Aedes aegypti* (L.) is the main vector of Dengue and yellow fever in the world. Since there is currently no vaccine that prevents Dengue infections (1-3), the Dengue virus control relies on controlling its mosquito vector. To assess the success of entomological interventions or surveillance of adult vector populations, several trapping systems have been evaluated. Recent advances in vector surveillance have generated a variety of traps for *Ae. aegypti*, such as Mosquito Magnet ™, BG-Sentinel ™ and Adultrap ™ (4-6).

The growing need for better surveillance devices has led to the design of several commercial prototypes that rely on a variety of stimuli. The design of Fay and Prince (7) and the Adultrap ™ use visual cues (8). Chemical cues are also used in several trap designs such as ovitraps (9), BG sentinel ™ trap (10). A combination of these factors are used in the Zumba® trap (11). Several field evaluations have reported that among the most successful devices produced so far are the Mosquito Magnet ™ (12) and BG Sentinel ™ (5, 13) which are considered nowadays to be the industry standards. One common feature of these devices is the collection of mosquitoes by means of suction mechanisms and the use of carbon dioxide as an attractant. It is a well-known fact, that mosquitoes are lured and attracted towards carbon dioxide sources (14). In addition, carbon dioxide has been shown to enhance the performance of light traps for the overall collection of mosquitoes

(15). It is therefore no surprising that some trap models rely on the use of CO_2 as an attractant. Most commonly, the semiochemicals are provided by a catalytic reaction in the case of Mosquito magnet ™ or by the use of pellets of dry-ice. Although these later commercially-available devices have shown very good results, their broad usage in third world countries has been hampered by their high initial purchase costs. Furthermore, there are often no suppliers of CO_2 cylinders nor are there dry-ice suppliers in endemic areas, and even when available these consumables are expensive in the long-term. A novel and cheaper strategy would be the use of yeast-generated CO_2. Previous studies on triatomine bugs have reported that live yeast cultures produce CO_2 which attracts nymphal stages (16-18). To the best of our knowledge, only Saitoh *et al*. (19), have reported the production and use of yeast-generated CO_2 *Aedes* spp. traps in the field. The objectives of the present study were two-fold: (1) to design and to test several prototypes of traps made from inexpensive materials; and (2) to evaluate the effectiveness of these traps under laboratory and semi-field conditions.

MATERIALS AND METHODS

Mosquito Rearing. Bioassays were carried out using a laboratory strain of *Ae. aegypti*, which has been kept at insectary facilities of Laboratorio de Entomología Médica, Universidad Autónoma de Nuevo León (FCB-UANL) since 2006, with a regular re-supply of field-collected eggs. Strips of filter paper which had eggs deposited onto its surface were placed into a plastic trays containing dechlorinated water. After the hatching of larvae, we added a sprinkle of powdered yeast to the water. Later, larvae were fed with Wardley™ fish food flakes (The Hartz Mountain Co., Secaucus, NJ, USA). Pupae stages were manually isolated into plastic cups and adult emergence was monitored frequently. Experimental mosquitoes were maintained in 30 cm^3 cages and were fed *ad libitum* with a 10% sugar solution.

Yeast mixture preparation. The attraction yeast mixture was prepared by mixing 200 ml of water, 50 g of sugar and 1 g of baker´s dry yeast *Saccharomyces cereviseae* (Levadura Azteca™, S. A. de C.V. México, D.F.). In the present study, we mixed the components using different proportions than described by Saitoh *et al*. (19). We used a first bottle named (A) containing 750 ml water, 75 g sugar and 6 g dry yeast; whereas, in a second bottle (B), the mixture contained 850 ml water, 50 g sugar and 3 g dry yeast. In the present study, we made our attraction mixture by dissolving the sugar powder completely in the water. Dry yeast was then carefully added until achieving a homogenous mixture. Description of trap prototypes. In the first part of our study, we designed and evaluated four different trap prototypes for laboratory testing, as follows.

Trap with a Top Funnel Entrance (TTFE). This model consisted of two assembled components. The upper part was a black straight-walled container made out of polyethylene terephthalate (PET), with a volume of 650 ml. The bottom of this container was removed and then it was positioned upside down, so that the removed bottom became the upper part. The hole of this upper part was replaced by a black funnel made out of a piece of ethylene vinyl acetate (EVA), whose narrow end was positioned in towards the inside of the container. The lower part of the trap was a clear PET container of a volume of 200 ml. This lower container was fixed to the upper inverted black container using two screw-type lids whose flat sides had been glued together. The center of these two glued lids had been cut off and the hole was covered with a fine fabric mesh to allow carbon dioxide to move into the upper chamber. The mesh barrier also prevented caught mosquitoes from entering the lower chamber containing the attraction mixture (**Figure 1A**).

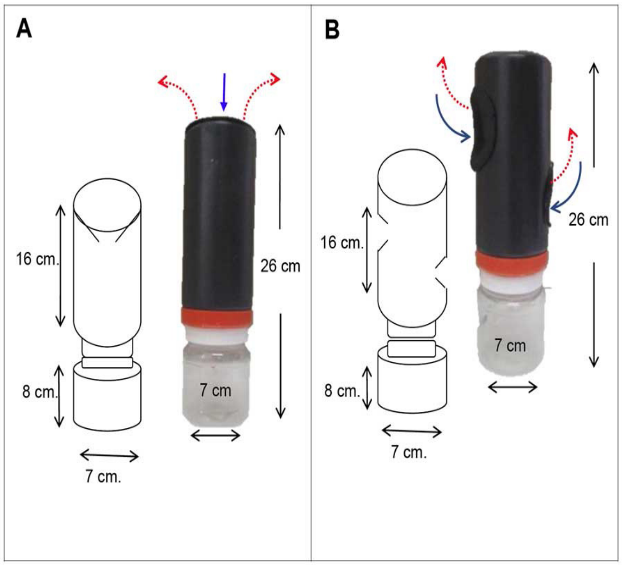

Figure 1. Photographs and schematic representation of two different designs of trap prototypes, which were evaluated under laboratory conditions.
A) Trap with Top Funnel Entrance (TTFE)
B) Trap with Side Funnel Entrances (TSFE). Dotted red lines represent the air flow of carbon dioxide, whereas, solid blue lines represent the direction of entrance of mosquitoes into the traps. Of these prototypes, only TSFE was evaluated under laboratory and semi-field conditions

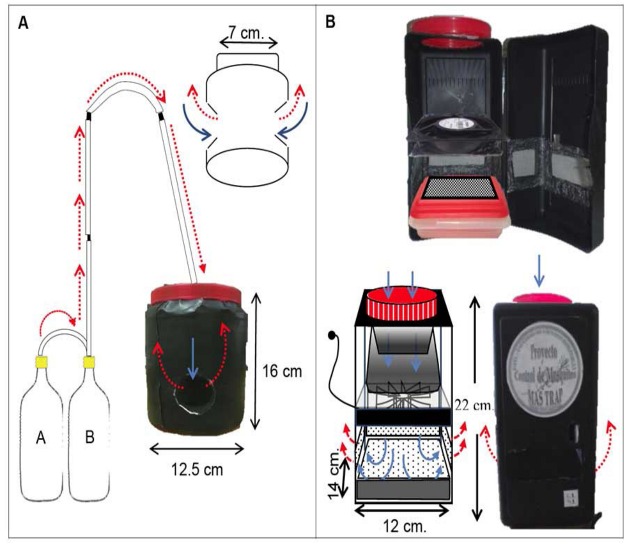

Figure 2. Photographs and schematic representation of two different designs of trap prototypes, which were evaluated under laboratory and semi-field conditions.
A) Trap Jar with Side Funnel Entrances (TJSFE)
B) Trap Mosquito Box (TMB). Dotted red lines represent the air flow of carbon dioxide, whereas, solid blue lines represent the direction of entrance of mosquitoes into the traps

Trap with Side Funnel Entrances (TSFE). The design of this trap was almost identical to the previous one, except that two funnel entrances made of the same (EVA) material were located on the sides of the upper black inverted PET container (**Figure 1B**).

Trap Jar with Side Funnel Entrances (TJSFE). This trap was made of a 1.5 l plastic PET jar which was completely covered with stripes of black non glossy thin cardboard. Three funnels made out of EVA black material, were inserted into the plastic jar. Based on the design of Saitoh *et al*. (19), we used a set of two plastic 2 l PET bottles. The system of the two bottles used by Saitoh *et al*. (19) consisted in one bottle (A) producing carbon dioxide which is conducted into a second bottle (B) and from this bottle, the gas is directed by means of a plastic tubing (ID= 7 mm) into the plastic jar bearing the side-positioned funnels (**Figure 2A**).

Trap Mosquito Box (TMB). This trap was constructed from a standard 12 x 14 x 22 cm black plastic box that is typically used to store index cards. The box was positioned in such a way that one of its sides became the bottom part. In the upper top, we removed a circular portion and a plastic screw lid was glued onto the edges of the hole. This screw lid acted as a fixing mechanism for the mosquito collection bag which was manufactured using a piece of black fine fabric. In the middle part and inside of the trap, we fixed a four inch diameter square-framed 117 AC Volt electric fan (Electrónica Steren™ S.A. de C.V, México, D.F.) operating at 2500-3000 rpm. The electrical fan was employed to help dispersion of carbon dioxide outside the trap, to reach farther distances. On the bottom part of the trap, we introduced a standard sandwich-type container with an air-tight lid. The lid was modified by cutting out a square portion, which was later covered by a plastic mesh to allow carbon dioxide to diffuse upwards. Approximately

2.5 cm above the bottom container, and on each of the four sides of the box, we removed a rectangular portion of the walls in order to create dispersion windows which were covered with a fine mesh fabric (**Figure 2B**).

Laboratory evaluation of trap prototypes. The first phase of the evaluation was carried out in an insectary room set at a temperature of 29°C and 50% relative humidity. Four cages of 60 cm^3 were placed on laboratory benches and were situated at least 30 cm from each other. Following this design, we were able to simultaneously test the response of *Ae. aegypti* females to all of the trap prototypes. In each experimental run we used a batch of 25 female *Ae. aegypti* mosquitoes of three-day old. Each trap prototype containing the attraction yeast mixture was positioned in the middle of the cages and was exposed for a period of 24 h. After this exposure period, traps were carefully taken out from the cages and were then chilled in a freezer for a few minutes to allow extraction and counting of captured mosquitoes. Female mosquitoes that were not captured by the prototypes were then aspirated from the evaluation cages and their numbers were recorded. Bioassays were replicated five times for each trap prototype, and in each replicate we used a new batch (n=25) of experimental mosquitoes.

Semi-field evaluation of trap prototypes. We selected the following trap prototypes: TSFE, TJSFE and TMB for semi-field use based on the previous laboratory trials. A fourth treatment in this evaluation was a TMB prototype supplemented with 10 ml (dilution 1:1) of reagent grade lactic acid (J. T. Baker™, Xalostoc, México). The yeast attraction mixture used in the bioassays was exactly the same as in the laboratory tests. Evaluation of trap prototypes was conducted in a yard located next to a greenhouse facility of the Facultad de Ciencias Biológicas at the Universidad Autónoma de Nuevo León. For the evaluation, we used four experimental cages of 2 m^3 each, constructed from polyvinyl chloride (PVC) tubing. Pieces of PVC tubing were bound together using PVC corner tees (**Figure 3**). Mosquito meshes made out of fine screen fabric were attached to the corners and middle points of the PVC frame. In each experimental cage, we released one-hundred female *Ae. aegypti* mosquitoes with an age of three days and which had not had access to a bloodmeal. Each trap prototype was positioned at the middle bottom of the cage and was left operating with the attraction yeast mixture for a 24 h period. From previous experiences we learned that small ants could gain access to the collection bag of traps and eat captured mosquitoes. To prevent this from happening, trap prototypes were placed onto polystyrene blocks and were isolated from the ground using water traps. After the 24 h period, traps and collection devices were taken to the laboratory and were chilled in a freezer for a few minutes. Later, each collection bag containing captured mosquitoes was emptied onto a chill-table (Bioquip Products™, Inc; Rancho Dominguez, CA, USA); this allowed for the counting of the number of mosquitoes captured per trap. Mosquitoes that were not captured by traps were aspirated from the cages and the numbers

Figure 3A. Experimental cages in semi-field trials. B. Traps were isolated to avoid damage by predators. C. Mosquitoes extraction was carried out with the backpack aspirator

were also recorded. Semi-field bioassays were replicated 15 times, using a new batch (n= 100) of mosquitoes for each replicate.

Data analyses. For each set of results, we tested if the criteria for the normality of data had been met using Anderson-Darling´s test (Minitab™ v. 11.0, Minitab Inc., Coventry, UK). In some cases we needed to transform data (x+1) to normalize the distributions. When this assumption could not be achieved, we analyzed data using a non-parametric test. Laboratory evaluation of trap prototypes was tested using a one-way ANOVA considering that response variable was the number of mosquitoes caught for each trap prototype. Post-hoc comparisons of means were carried out using Tukey´s test. Semi-field results were analyzed using non-parametric analysis of variance known as Kruskal-Wallis´ (H) test, and the response variable was also the number of female *Ae. aegypti* collected for each prototype after 24 h. A non-parametric Nemenyi´s multiple comparison test was also calculated to reveal differences among the means. All statistical tests were considered significant if $p< 0.05$.

RESULTS

Laboratory evaluation of trap prototypes. In the laboratory we evaluated four prototypes of traps for *Ae. aegypti* mosquitoes and found that only TMB had a capture effectiveness up to 94%, which represented almost a 3-fold difference with respect to the other designs. The remaining three prototypes (TTFE, TSFE, and TJSFE) elicited lower responses to female *Ae. aegypti*. It was found by ANOVA that there were significant differences between the four treatments ($F= 14.02$; d.f. = 3,16; $p<0.01$) and post hoc comparisons using Tukey´s test ($q = 4.05$; d.f. = 4,16; $p<0.05$) showed that TMB had a significant different mean response that those mean responses obtained with TTFE, TSFE and TJSFE. Likewise, TSFE and TTFE were found to be equally effective in their mean capture of mosquitoes. Prototype TJSFE had the lowest of all treatments in mean of mosquito collection rates (**Table 1**).

Semi-field Evaluation of Trap Prototypes. During the semi-field evaluation of prototypes, it was found that female *Ae. aegypti* mosquitoes were mainly attracted to prototypes TMB and TMB supplemented with lactic acid (Kruskal-Wallis $H= 45.97$;d.f.= 3, $p<0.05$). A non-parametric test for multiple comparisons (Nemenyi´s test $q= 3.76$; d.f.= 4,56; $p<0.05$) revealed that mosquitos response to TMB and TMB supplemented with lactic acid was significantly different to prototypes TSFE and TJSFE. Moreover, the mean mosquito response obtained with TMB and TMB+lactic acid was at least four-times higher than that obtained with TSFE and TJSFE designs (**Table 1**).

DISCUSSION

It is clear from the first set of experiments in the laboratory, that one design (TMB) performed significantly better at attracting and collecting female *Ae. aegypti*. Those prototypes involving a passive system for the diffusion of carbon dioxide molecules had the lowest mean capture rates. The trap prototypes TTFE, TSFE and TJSFE did not collect as many mosquitoes as compared with TMB prototype. The are two possible explanations as to why mosquitoes were not attracted to such models: (1) all these models rely on passive diffusion systems to disperse CO_2 produced by yeast. It is possible that these passive systems do not allow the attractant material to travel far from the trap (2). It is not known whether the funnel entrances on these traps were wide enough or if they had been positioned in a most facilitating way to allow mosquitoes to gain access to the traps. In contrast, our TMB prototype that utilizes an electrical fan that disperses carbon dioxide to greater distances, and was by far the most effective trap to collect *Ae. aegypti* mosquitoes. It is very likely that the addition of an electric fan provided a more efficient mechanism to disperse the attractant carbon dioxide and perhaps the fan created an odor

Table 1
Mean response (±SD) of Ae. aegypti mosquitoes to four prototypes of traps evaluated under laboratory and semi-field conditions

Evaluation	PROTOTYPES OF TRAPS					N	n	Test value	P value
	TTFE*	TSFE†	TJSFE‡	TMB§ / CO_2	TMB + Lactic acid				
Laboratory	7.6 (±6.8)[b]	8.8 (±4.97)[b]	1.4 (±0.89)[c]	23.6 (±1.14)[a]	-	5	25	F = 14.02	< 0.05
Semi-field	-	13.9 (±3.7)[b]	27.4 (±20)[b]	97.4 (±2.1)[a]	94.9 (±5.2)[a]	15	100	H = 45.97	< 0.05

* TTFE = Trap with a Top Funnel Entrance
† TSFE = Trap with Side Funnel Entrances
‡ TJSFE = Trap Jar with Side Funnel Entrances
§ TMB = Trap Mosquito Box
Means followed by the same letter are not significantly different
N = Refers to the number of replicates per bioassay
n = Refers to the number of experimental mosquitoes per replicate

plume that traveled to greater distances and in all directions.

Some laboratory studies with olfactometers usually have used CO_2 as a constant air-flow to attract mosquitoes, however it is very likely that under field conditions, a constant air-flow does not occur and that most probably, the molecules of attractive CO_2 travel in the odor plume as undiluted packets or puffs of air. For instance, Dekker *et al*. (20) showed that *An. gambiae* mosquitoes were more attracted to turbulent air containing carbon dioxide than to a homogeneous airflow. Regarding our results, we observed that the addition of an electric fan to the TMB model, significantly increased the mean number of mosquitoes caught and perhaps this improvement could be related to the creation of an artificial odor plume conveying the yeast-produced CO_2. In Saitoh *et al*. (19) paper, it was shown that mosquitoes were effectively captured with the use of two CO_2 producing bottles which conducted the attractive gas to a releasing bottle positioned next to a suction device. This collection device had a down-draft fan, and it is possible that the suction device might also act as dispersion mechanism for the attractive carbon dioxide. In general terms we can suggest that in addition of an attractant material like the carbon dioxide produced by yeast, other factors such as dispersion mechanisms will help to obtain better results in collecting *Ae. aegypti* mosquitoes.

The semi-field experiments described in this paper shown that TMB prototype was at least three times more effective in collecting the experimental mosquitos and that the addition of lactic acid did not enhance the mean number of mosquitoes caught. The good results of this semi-field evaluation of the TMB prototype prompted us to design a field evaluation (data not included in this paper) of our trap against two standard collection methods (backpack aspirator™ and BG Sentinel™), finding that under field conditions TMB prototype had a better performance that the backpack aspirator™ and that TMB was equally effective as the BG-Sentinel™ (21). Low-costs technologies to design traps are always required in endemic third-world countries and therefore we suggest that more studies are needed in this area. Finally, we concluded that out TMB is a very good option to implement in surveillance programs because of its low cost and its simplicity to operate under field conditions.

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