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NEW TOOLS FOR SURVEILLANCE OF ADULT YELLOW FEVER MOSQUITOES: COMPARISON OF TRAP CATCHES WITH HUMAN LANDING RATES IN AN URBAN ENVIRONMENT

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ABSTRACT. A novel mosquito trapping system, the BG-Sentinel® trap, was evaluated as a monitoring tool for adult *Aedes aegypti* in field tests in the city of Belo Horizonte, Brazil. Human landing/biting collections, a gas-powered CO_2 trap, and a Fay-Prince trap with only visual cues serving as references to evaluate the efficacy of the new trap. The BG-Sentinel is a simple suction trap that uses upward-directed air currents as well as visual cues to attract mosquitoes. The trap was tested with a new dispenser system (BG-Lure®) that releases artificial human skin odors and needs no CO_2. In comparison with the two other traps, the BG-Sentinel caught significantly more *Ae. aegypti.* Although human landing rates were the highest, there was no significant difference between human landing rates and the capture rates of the BG-Sentinel trap. This finding indicates that the trap can be considered as an acceptable alternative to human landing/biting collections in the surveillance of adult host-seeking dengue vectors. The addition of the BG-Lure to the gas-powered CO_2 trap greatly increased its efficacy. This combination, however, was not significantly more effective than the BG-Sentinel without CO_2. In a 6-month comparison between the BG-Sentinel and a sticky ovitrap for gravid females, the BG-Sentinel proved to be a far more efficient and sensitive tool to measure the density of *Ae. aegypti* populations.

KEY WORDS *Aedes aegypti, Stegomyia aegypti,* surveillance, dengue, kairomones, traps

INTRODUCTION

Lack of efficient adult monitoring tools

Sampling and trapping devices for arthropod vectors of pathogens are important tools for the collection of ecological and behavioral data such as population densities, daily and seasonal abundance, vector distribution, or survivorship after control measures. These data are crucial in understanding the epidemic potential and in setting up early and effective control strategies. Depending on the objectives, the level of infestation, available funding, and the skill of personnel, the surveillance of *Aedes aegypti* (L.) (or *Stegomyia aegypti*; see Reinert et al. 2004) populations has traditionally relied upon labor-intensive methods such as the sampling of immature stages from breeding sites, the systematic search and collection of resting adults, or the use of ovitraps that detect ovipositing females through their eggs (WHO 1997, Morrison et al. 2004). A recent improvement is the sticky ovitrap that catches adults and therefore gives a more direct measure of the egg-laying portion of the mosquito population (Ritchie et al. 2003). These methods are also slow, requiring days to complete large surveys or to obtain results. In addition, the indices derived from these sampling methods often lack epidemiological sensitivity because the density and proportion of host-seeking adults is the crucial indicator of risk for transmission of diseases (Focks 2003). One method that addresses this point is the determination of landing rates of host-seeking females on human volunteers, which is labor-intensive, often inaccurate, and ethically questionable. Another method is the inspection of households by using backpack aspirators to suck in flying or resting mosquitoes (Morrison et al. 2004, Schoeler et al. 2004). Although this method results in the collection of high numbers of adult mosquitoes, the sampling is not specific for vectors, the inspection process is often considered an intrusion of private life, and results may vary with the skills and motivation of the inspectors.

An alternative could be traps that catch host-seeking females. Several types of trapping devices have been tested in the field, but their efficacy and sensitivity have not been very satisfactory for *Ae. aegypti* (Canyon and Hii 1997, Jones et al. 2003, Schoeler et al. 2004, Russell 2004). Besides a low performance, a major drawback of such trapping devices is that they often require CO_2, the most widely used attractant for host-seeking female mosquitoes. However, the consumption of CO_2 is high—about 1 kg/day—and providing such amounts either by using gas cylinders, dry ice, or by the combustion of propane causes logistical problems and high costs. Hence, there is a need for an efficient and simple non-CO_2-based trapping method for *Ae. aegypti* that can give a quick, realistic, and standardized estimation of the prevalent adult mosquito population that is directly involved in the transmission of disease agents.

Background of new trapping technology: New promising trapping technologies have recently been developed by BioGents GmbH, a spin-off company from the Institute of Zoology at the University of Regensburg, Regensburg, Germany. Results from the Institute's research in sensory ecology produced a new blend of mosquito attractants consisting of lactic acid, ammonia, and caproic acid, substances

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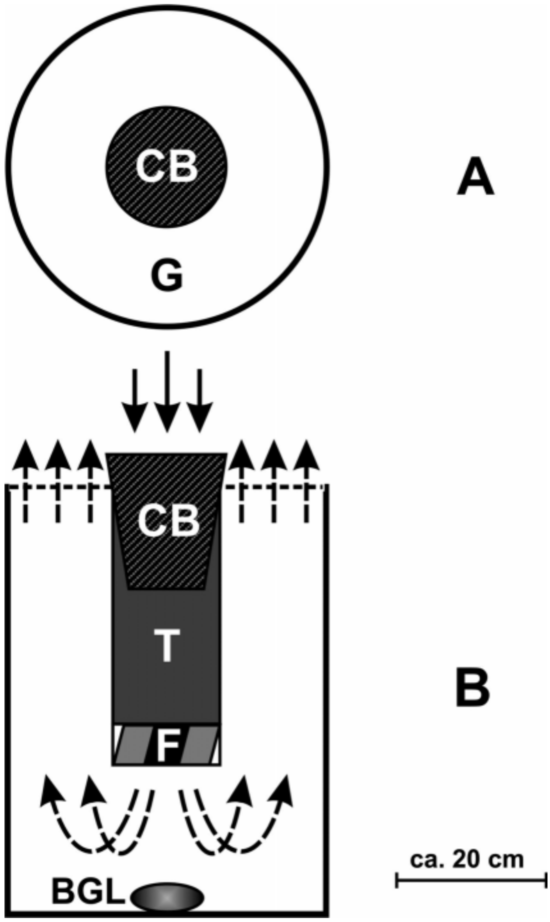

Fig. 1. Functional diagram of the BG-Sentinel mosquito trap. (A) View from above. CB, catch bag; G, white gauze cover. (B) Longitudinal section of the trap. T, black tube; F, fan; BGL, BG-Lure. Arrows indicate the direction of the airflow.

that also are found on human skin. The blend is constantly emitted in a fixed ratio from a long-lasting dispenser, the BG-Lure®. In addition, the BG-Sentinel®, a new trapping device, has been developed that 1) attempts to mimic convection currents created by a human body, 2) uses visual cues intended to be attractive, and 3) releases the skin compounds from the BG-Lure through a large surface area (Fig. 1).

Objectives: The aim of this study was to field test the BG-Sentinel trap and BG-Lure in an urban environment, where the density of human hosts is high and outbreaks of dengue are likely to occur, and to see whether the trap can serve as a new surveillance method for collecting high numbers of adult *Ae. aegypti* without using CO_2 as an attractant. Because the efficacy of traps is difficult to assess without relevant comparison standards, human landing/biting collections, a Fay-Prince trap (a CDC-type suction trap that uses additional visual cues to attract mosquitoes from a distance), and the Mosquito Magnet Liberty (MML; a trap that produces warm and humid CO_2 from the combustion of propane or butane) were included in two separate experiments. In an additional experiment, the efficacy of the BG-Sentinel was compared with that of a sticky ovitrap.

MATERIALS AND METHODS

Study site

All experiments were conducted between October 2003 and April 2004 in Belo Horizonte City (900-m altitude; 19.9°S, 43.9°W) in Minas Gerais State, Brazil, with a population of more than 2 million. The rainy season is usually from November to April and typically features a higher density of *Ae. aegypti*. The climate is tropical, with annual average daily temperature of 25°C and the highest level of precipitation occurring in January with approximately 296 mm. The campus of the Institute of Biological Sciences at the Federal University of Minas Gerais was chosen as the study site because of high populations of *Ae. aegypti* and large numbers of human hosts, because of every day university life and the social activities of students, staff, and personnel on the campus. All collection sites were outdoor locations, partly covered by the roofs of the surrounding buildings.

Traps evaluated: Traps evaluated in this study included the BG-Sentinel Trap, the bidirectional Fay-Prince trap, and the MML trap. Human landing collections were used as a reference against which the efficacy of the other traps was compared. In another long-term experiment, a sticky oviposition trap was used as a reference collection method for gravid adult females.

The BG-Sentinel Trap consists of an easy-to-transport, collapsible white bucket with white gauze covering its opening. In the middle of the gauze cover, there is a black tube through which a down flow is created by a 12-V d.c. fan that causes any mosquito in the vicinity of the opening to be sucked into a catch bag. The catch bag is located above the suction fan, thereby avoiding damage to specimens passing through the fan. The air then exits the trap through the large surface of white gauze (987 cm^2): the design therefore generates ascending currents (Fig. 1). These currents are similar to convection currents produced by a human host in their direction, geometrical structure, and, because of the addition of attractants, also in their chemical composition. The attractants are given off by the BG-Lure (BioGents GmbH), a dispenser that releases a combination of lactic acid, ammonia, and caproic acid, all substances that are found in human skin (Geier et al. 1999, Bosch et al. 2000). The dispenser emits the attractants for up to 5 months. During the tests, the BG-Sentinel traps were simply placed on the ground.

The bidirectional Fay-Prince trap (John W. Hock Co., Gainesville, FL) was originally designed to catch *Ae. aegypti* (Fay and Prince 1970). The trap is based on the attractive properties of contrasting black and white panels. Attracted insects are drawn into a catch bag through a fan. No additional attractants were used. The trap was hung 1.5 m above the floor. This position represents normal use of the trap.

The MML trap (American Biophysics Corp., East Greenwich, RI) uses a catalytic combustion unit to convert propane or butane gas into a warm and humid CO_2 stream. In this study, a portable butane tank was used as a gas source because propane is difficult to obtain in Brazil. The average consumption of butane was about 0.5 kg/day. The fan of this trap model is powered by a 12-V d.c. power supply. Two models of this trap type were used in different experiments. One model was later equipped with the BG-Lure, which was inserted into the black exhaust tube through which the trap releases CO_2 and water vapor. No additional attractants were added to the other model.

As a sticky ovitrap, the Mosquitrap® (Ecovec Ltda, Belo Horizonte, Brazil) was used. The trap is similar to a trap type described by Ritchie et al. (2003). The trap is essentially a round, black container with a volume of 1.2 liters. The trap is 16 cm in height and has an opening of 7 cm in diameter at the top. The lower half of the trap was filled with a 10% diluted hay infusion (100 ml of prepared infusion in 900 ml of water), protected by a plastic mesh to keep mosquitoes from reaching the surface. The prepared infusion was made by steeping 20 g of dry grass hay in 1 liter of tap water for 7 days in a tightly closed plastic garbage can in a shaded outdoor location. A black sticky board (AgriSense-BCS Ltd., Pontypridd, United Kingdom) was fitted to the inside wall of the upper half of the container. The sticky board and hay infusion were changed at least each 2 weeks.

The human landing/biting rates were measured by volunteers. The volunteers, one at a time, sat on a chair, with exposed legs. The volunteers collected mosquitoes by themselves into glass vials by using a mouth-suction aspirator as they landed on their legs or arms. Because of the low landing rates, the mosquitoes could be always collected before they get a chance to bite.

Experimental procedure and data analysis

Four different experiments were conducted.

Experiment 1: This preliminary test evaluated the perormance of a BG-Sentinel trap in direct comparison with the MML trap. Two separate trials were conducted at 2 locations (Barbecue Station and Caixa Bank), which are about 100 m apart and separated by several buildings. At each location, a BG-Sentinel trap (including the BG-Lure) and a MML trap were placed at a distance of 15 m from each other. Sampling periods were 24 h. The positions of the 2 traps were exchanged so that each kind of trap alternated positions after every 24-h sampling period. Eleven tests were conducted at the Barbecue Station (December 10, 12, 15, 16, 17, 18, and 19, 2003 and January 7, 8, 9, and 12, 2004). Ten tests were conducted at the Caixa Bank (December 12, 15, 16, 17, 18, and 19, 2003 and January 7, 8, 9, and 12, 2004). Trapped insects were collected at the end of each sampling period. Mosquitoes were identified to species, and other insects were identified to genus or family. The data from each location were analyzed separately. Statistical differences between the 2 traps were assessed using the nonparametric Mann–Whitney *U*-test.

Experiment 2: This experiment consisted of a Latin square design, where 5 collection methods (human landing collection, BG-Sentinel with a BG-Lure, MML trap, MML trap with a BG-Lure, and a bidirectional Fay-Prince trap) were evaluated at 5 different outdoor locations (Botanical Department, Students' Office, ICB Building, Guard Booth, and Caixa Bank). Traps/collecting methods were rotated daily between the 5 locations until each trap/collecting method was tested once at every location. A trial started on Monday and was completed on Friday; four complete trials were conducted in four consecutive weeks from January 19, 2004 to February 17, 2004, yielding 20 trap days per treatment in total. Human landing collections were conducted for 3 h per day in alternating sampling periods (0700 to 1000 h, 1000 to 1300 h, 1300 to 1600 h, and 1600 to 1900 h). Every day, one human landing collection was conducted, and both the position and the time period of collection changed daily. Before and after the human landing collection, mosquitoes also were collected from the traps to determine the number of mosquitoes that were caught during this 3-h period. Thereafter, the traps remained active at each location until the next day, when traps and human landing collections were rotated, and a new test was started. Therefore, we conducted 2 separate analyses: One with all 5 trapping methods for the 3-h collection period corresponding to the human landing collections and another with the 4 mechanical traps for the remaining 21 h of each test period. Throughout the whole experiment, human landing collection were conducted by the same test person (female Caucasian, 30 years old; UK). Trap collection data for both collection periods (3 h and 21 h) were analyzed separately and are presented as raw data. All data were transformed to $\log(x + 1)$ before further statistical analysis. Trap and location effects were evaluated by using analysis of variance (ANOVA) (SPSS 12.0.1, SPSS Inc., Chicago, IL). Post hoc mean comparisons were performed using Tukey's mean separation procedure ($\alpha = 0.05$).

Experiment 3: This study was basically a repetition of experiment 2, 1 month later and with slight changes in the experimental design. Again, the 5 collection methods were evaluated at the same outdoor locations as for experiment 2 with a daily rotation of the treatments. Instead of the 4 trials in experiment 2, only 2 trials were conducted (March 15, 16, 17, 18, 19, 29, and 30 and April 5 and 6, 2004), yielding 10 trap days in total. Human landing rates were determined twice daily for 3 h each by 2 different test persons. In each of the 2 trials, different test persons were used: In trial 1, a 30-year-old female Caucasian (MB) collected from 0700 to 1000 and a male 20-year-old Caucasian (JL) collected from 1600 to 1900 h. In trial 2, UK collected from 1000 to 1300 h, and a human female Caucasian, 29 years old (AP) from 1300 to 1600 h. As in experiment 2, mosquitoes were collected from the traps before and after each human landing collection to check for the number of mosquitoes that were caught during these 3-h sample periods. After that, the traps remained active at each location until the next day when traps and human landing collections were rotated, and a new test was started. This procedure yielded a 20 periods of 3-h collections in total with all 5 treatments and 10 periods of 18-h collections in total with only the 4 trap treatments. The statistical analysis of the data followed the same procedure as described for experiment 2.

For additional analysis, the data from experiments 2 and 3 were pooled to examine the proportions of sampling periods when at least 1 *Ae. aegypti* mosquito was captured (positive trap index). For each of the total of 30 test days, 1 3-h sampling period was chosen to calculate the positive trap index for each trap/collecting method. Each trap type or collection method was compared by chi-square analyses. Significance was indicated by a *P* value equal to or less than 0.05.

Experiment 4: In this experiment, the efficacy of the BG-Sentinel trap was compared with a sticky ovitrap that captured ovipositing female mosquitoes. Both traps were placed at the same location (Barbecue Station) at a distance of 10 m from each other. From October 3, 2003 to April 6, 2004, 65 trap days in total were conducted. The individual collection periods were between 20 and 28 h. After every collection period, the locations of the traps were rotated to minimize the effects of site variation. To test for statistical differences between the two traps, the nonparametric Mann–Whitney *U*-test was used. Positive trap indices were calculated from the number of collection periods when at least 1 mosquito was caught and from the number of all collection periods. The positive trap indices for the 2 treatments were compared by chi-square analyses. Significance was indicated by a *P* value equal to or less than 0.05.

RESULTS

In total, 1,611 adult mosquitoes were collected by the combined sampling methods during the course of all the experiments. Of the mosquitoes collected, 68% (1,097) were female *Ae. aegypti*, 24% (384) were male *Ae. aegypti*, 7% (108) were female *Culex quinquefasciatus* Say, and about 1% (22) were male *Cx. quinquefasciatus.* During the daytime sampling periods, only *Ae. aegypti* was collected. Additional mosquito species were not found. Other insect species were occasionally trapped, depending on trap type, location, and time. Although these specimens were not quantified, flies such as *Drosophila* spp. or *Musca* spp. were found in the BG-Sentinel trap more often than in the other trap types.

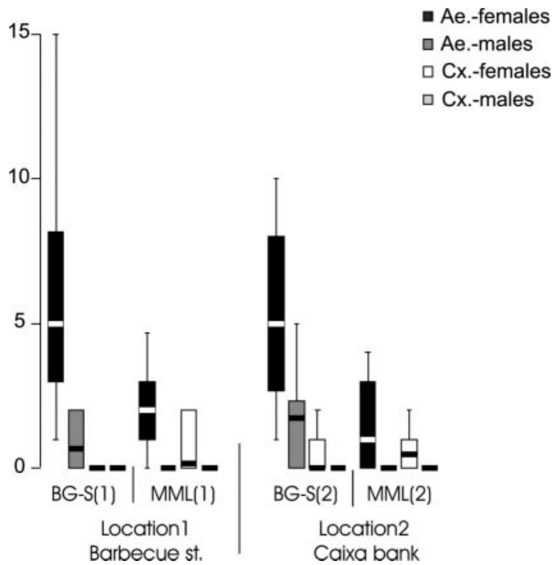

Fig. 2. Box-and-whisker plots of mosquitoes collected at 2 different locations with a BG-Sentinel trap with a BG-Lure dispenser (BG-S) and the MML trap, a CO_2-generating gas trap (MML). Numbers in parentheses show that two different models were used at the different test locations. Eleven tests were conducted at location 1, and 10 tests were conducted at location 2. Ae., *Ae. Aegypti*; Cx., *Cx. quinquefasciatus.*

Experiment 1: Fig. 2 shows the distribution of mosquitoes in the BG-Sentinel trap and the MML trap at the 2 test locations. At both locations, the BG-Sentinel trap collected significantly more female *Ae. aegypti* than the MML trap (location 1: Mann–Whitney *U*-test: 23.5, $Z = -2.242$, $P = 0.025$; location 2: Mann-Whitney *U*-test: 19.5, $Z = -2.320$, $P = 0.02$). The BG-Sentinel trap also caught more male *Ae. aegypti,* but the difference was only significant at location 2 (Mann–Whitney *U*-test: 19.5, $Z = -2.607$, $P = 0.009$). Because of their low numbers, no significant differences could be observed for both *Cx. quinquefasciatus* males and females. There was, however, a tendency that the MML trap caught more *Cx. quinquefasciatus* than the BG-Sentinel. When the trapping results of both locations are pooled, 135 female *Ae. aegypti,*

Table 1. Mosquitoes collected by 5 different collection methods during 3-h sampling periods in experiment 2.

Adult *Ae. aegypti*		Type of collection: BG-Sentinel + BG-Lure	MML with CO_2	MML with CO_2 + BG-Lure	Fay-Prince (no lure, no CO_2)	Human landing collection
Females	Total collected	30	2	33	3	47
	Mean/trap/3 h[1]	1.5b	0.1a	1.7b	0.2a	2.3b
	SEM	0.44	0.07	0.36	0.12	0.59
Males	Total collected	5	4	9	2	44
	Mean/trap/3 h	0.3a	0.2a	0.5a	0.1a	2.2b
	SEM	0.16	0.12	0.42	0.07	0.44
	Trap days	20	20	19	19	20

[1] Mean/trap/3 h, mean number of mosquitoes caught during the 3-h sampling periods; number of collections, number of 3-h collections when traps worked without technical problems.

38 male *Ae. aegypti,* 8 female *Cx. quinquefasciatus,* and 1 male *Cx. quinquefasciatus* were collected with the BG-Sentinel. The MML trap caught 72 female and 7 male *Ae. aegypti* in total as well as 24 female and 7 male *Cx. quinquefasciatus.*

Experiment 2: In this experiment, human landing rates were compared with trap collections from the BG-Sentinel, the MML trap, the MML trap with an additional BG-Lure, and the Fay-Prince trap. Table 1 summarizes the results of the 3-h sampling periods. Only *Ae. aegypti* adults were collected. ANOVA analysis showed significant differences between treatments both for the number of female ($F = 11.005$, df 4, $P < 0.001$) and male ($F = 14.416$, df 4, $P < 0.001$) *Ae. aegypti.* The mean numbers of female *Ae. aegypti* collected per sampling period by the human volunteer, the BG-Sentinel trap, and the MML trap with BG-Lure (2.3, 1.5, and 1.7, respectively) were significantly higher than that of the MML (0.1) and Fay-Prince traps (0.2), but no significant difference was found between the 1st 3 collection methods. Human landing collections were significantly more effective for collecting male *Ae. aegypti* than any other trapping method. No significant difference was observed between the number of males caught in the traps. Figure 3 shows the number of mosquitoes collected with all sampling methods combined, during the 4 different time windows. In the morning (0700 to 1000 h), significantly fewer female *Ae. aegypti* were caught than in the late afternoon (1600 to 1900 h) (ANOVA: $F = 3.498$, df 3, $P = 0.019$; Tukey's post hoc: $P = 0.01$).

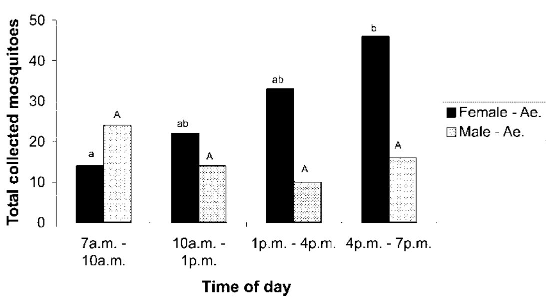

Fig. 3. Number of *Ae. aegypti* collected during the different 3-h sampling periods of experiment 2. Bars with the same letters are not significantly different ($P > 0.05$).

Because the trap devices remained active after the 3-h sampling period until the new human landing collection the next day, the collections of the 4 trap devices could be analyzed as well for these 21-h sampling periods. The results are shown in Table 2. As in the 3-h sampling periods, ANOVA demonstrated that the type of trap used was a significant factor in the number of *Ae. aegypti* collected (ANOVA: females, $F = 24.336$, df 3, $P < 0.001$; males, $F = 8.170$, df 3, $P < 0.001$). For *Cx. quinquefasciatus,* however, no significant differences could be observed. Figure 4 shows combined catches at each test location: there was no significant difference for either mosquito species or sexes for the total number of mosquitoes collected in all collection periods at the different locations.

Experiment 3: This experiment was essentially a repeat of experiment 2 with slight modifications to the experimental design (see Materials and Methods). Table 3 summarizes the results of the 3-h sampling periods with the BG-Sentinel, the MML trap, the MML trap with a BG-Lure, and the Fay-Prince trap. As in experiment 2, only *Ae. aegypti* were collected, but the numbers were generally lower. ANOVA showed significant differences between treatments for the number of female ($F = 12.451$, df 4, $P < 0.001$) and male ($F = 5.951$, df 4, $P < 0.001$) *Ae. aegypti* collected. The mean numbers of female *Ae. aegypti* collected per sampling period was highest for human landing collection (1.95) followed by the MML trap with a BG-Lure (1.26) and the BG-Sentinel trap (0.75). Only a few females were caught by the MML and Fay-Prince traps. The mean sampling rates did not differ significantly between the human volunteer and the MML trap with the BG-Lure, but the difference between the human landing collection and the BG-Sentinel trap was significant. There were no significant differences in the mean sampling rates of *Ae. aegypti* between human landing collection, the BG-

Table 2. Mosquitoes collected by 4 different trapping devices during 18-h sampling periods in experiment 2.

Species/sex		BG-Sentinel + BG-Lure	MML with CO_2	MML with CO_2 + BG-lure	Fay-Prince (no lure, no CO_2)
		Type of collection			
Ae. aegypti females	Total collected	92	24	159	6
	Mean/trap/21 h[1]	4.6b	1.3a	8.4b	0.3a
	SEM	0.79	0.40	1.82	0.16
Ae. aegypti males	Total collected	27	12	52	1
	Mean/trap/21 h	1.3bc	0.7ab	2.7c	0.1a
	SEM	0.33	0.34	0.82	0.06
Cx. quinquefasciatus females	Total collected	5	6	7	0
	Mean/trap/21 h	0.3a	0.3a	0.4a	0.0a
	SEM	0.18	0.16	0.19	0.00
Cx. quinquefasciatus males	Total collected	0	1	0	3
	Mean/trap/21 h	0.0a	0.1a	0.0a	0.2a
	SEM	0.00	0.06	0.00	0.12
	Trap days	20	18	19	18

[1] Mean/trap/18 h, mean number of mosquitoes caught during the 18-h sampling periods; number of collections, number of days when traps worked without technical problems.

Sentinel trap, and the MML with the BG-Lure. The Fay-Prince trap and the MML traps, however, did significantly differ in their mean collecting rate for male mosquitoes. In this experiment, human landing collections were conducted by 4 volunteers. Although some persons yielded slightly higher numbers of female or male *Ae. aegypti* collected, ANOVA did not reveal any significant differences. In contrast with the results from experiment 2, the numbers of collected mosquitoes did not differ significantly according to the time of day when the samplings were conducted (Fig. 5). In this experiment, 2 3-h sampling periods were conducted per day. The trap devices remained active between and after these sampling periods until the next human landing collection on the following day, giving a remaining collection period of 18 h for the 4 trap devices. The results are summarized in Table 4. Significant differences between the trapping devices could only be observed for female *Ae. aegypti* (ANOVA: $F = 12.443$; df 3, $P < 0.001$). The BG-Sentinel trap and the MML trap with the BG-Lure collected almost equal numbers of females, with

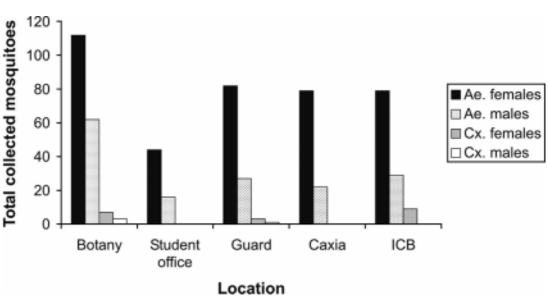

Fig. 4. Total number of mosquitoes collected at the different test locations in the course of experiment 2. Ae., *Ae. Aegypti*; Cx., *Cx. quinquefasciatus*. Bars with the same letters are not significantly different ($P > 0.05$).

means of 2.56 and 2.60 mosquitoes collected, respectively, per sampling period. Both means were significantly different from the mean collections of the MML trap and the Fay-Prince trap. As in experiment 2, no significant effect of trap location could be observed (Fig. 6).

The data from all test days of experiments 2 and 3 were pooled to find out which collection method was most sensitive in detecting the presence of adult *Ae. aegypti* in the test area. Figure 7 shows the percentage of positive 3-h collection periods (i.e., periods in which at least 1 mosquito was collected) for the BG-Sentinel trap, the MML trap, the MML trap with a BG-Lure, the Fay-Prince trap, and a human volunteer. Human landing collections yielded females in 70% of the total sampling periods. The MML trap with BG-Lure and the BG-Sentinel trap caught females in 67% and 53% of the sampling periods, respectively. These percentages were not significantly different from that of the human landing collection. Significantly lower percentages were found with the MML trap (10%) and the Fay-Prince trap (7%). For males, the human landing collection yielded 73% positive sampling periods. This percentage was significantly higher than the percentages for any of the traps (Fig. 7).

Experiment 4: In this experiment, a 6-month comparison between the BG-Sentinel trap and a sticky ovitrap was conducted at the same location, giving 65 trap days in total with a mean sampling duration of 24 h. The BG-Sentinel trap caught 469 adult *Ae. Aegypti* in total, 128 males and 341 females. Other insects, particularly fruit flies (Drosophilidae) and house flies (Muscidae) also were caught but not quantified. The sticky ovitrap caught 16 adult *Ae. Aegypti* in total, all of which were females. Occasionally, other insects and spiders were caught but not quantified. Figure 8 shows the number of mosquitoes collected for each sampling

Table 3. Mosquitoes collected by 5 different collection methods during 3-h sampling periods in experiment 3.

Adult *Ae. aegypti*		BG-Sentinel + BG-Lure	MML with CO_2	MML with CO_2 + BG-Lure	Fay-Prince (no lure, no CO_2)	Human landing collection
		Type of collection				
Females	Total collected	15	2	24	1	39
	Mean/trap/3 h[1]	0.75ab	0.10a	1.26bc	0.05a	1.95c
	SEM	0.29	0.07	0.31	0.05	0.43
Males	Total collected	9	1	10	0	19
	Mean/trap/3 h	0.45ab	0.05a	0.53ab	0.00a	0.95b
	SEM	0.20	0.05	0.18	0.00	0.26
	No. of collections	20	20	19	19	20

[1] Mean/trap/3 h, mean number of mosquitoes caught during the 3-h sampling periods; trap days, number of days when traps worked without technical problems.

day. The mean numbers of female *Ae. aegypti* collected per day were significantly different with 5.25 ± 0.76 (±SEM) for the BG-Sentinel trap and 0.25 ± 0.07 for the sticky ovitrap (Student's *t*-test for paired samples: $t = 6.627$, df 64, $P < 0.001$). The maximum number of collected females per day was 27 for the BG-Sentinel trap and 2 for the sticky ovitrap.

The BG-Sentinel trap detected at least 1 female *Ae. aegypti* in 88% of the total sampling days, compared with 18% for the sticky ovitrap. These proportions are significantly different (chi-square: $P <$ 0.001), indicating a much higher sensitivity of the BG-Sentinel trap for detection of adult female *Ae. aegypti.*

DISCUSSION

The results demonstrate that the BG-Sentinel trap has the potential to be a versatile, simple, and highly sensitive surveillance tool for *Ae. aegypti,* the most important vector for dengue. The BG-Sentinel trap can be used as a quick detector for the presence of *Ae. aegypti*: no significant difference in the sensitivity for detection of adult females could be observed between the 3-h sampling periods of the BG-Sentinel trap, the human landing collection, and the MML trap plus BG-Lure, although the collection rate for the BG-Sentinel trap was slightly

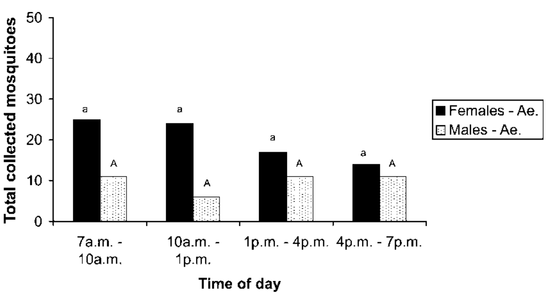

Fig. 5. Number of *Ae. aegypti* collected during the different 3-h sampling periods of experiment 3. Bars with the same letters are not significantly different ($P > 0.05$).

lower (Fig. 7). Because the BG-Sentinel needs no CO_2 and the attractant remains active for several months, the trap also can serve for the long-term monitoring of mosquito population density (Fig. 8).

Comparison with conventional adult collection methods for Ae. aegypti

The combined experiments in this study show that the BG-Sentinel trap/BG-Lure system was about 1/2 as efficient as a human volunteer in collecting adult female *Ae. aegypti.* Other adult traps tested were significantly less efficient than the BG-Sentinel trap (i.e., the unidirectional Fay-Prince trap with only visual cues and the MML trap, which produces only warm and humid CO_2 from gas combustion) or reached the same efficiency only when the BG-Lure was added (the MML trap plus BG-Lure).

Previous studies have demonstrated that conventional surveillance traps, even with the addition of CO_2, are not very sensitive for the detection of *Ae. aegypti* and do not provide an alternative to measuring human landing rates. Canyon and Hii (1997) field-tested lactic acid, octenol, and CO_2 as additional attractants in bidirectional Fay-Prince traps. The only trap that actually caught *Ae. aegypti* in the field was the trap with CO_2 (1.8 female and male mosquitoes in 14 h, i.e., 0.1 mosquito/h), compared with mean human landing rates of between 1.7 and 3.6 in 10 min. In a field study in Thailand, Jones et al. (2003) tested an omnidirectional Fay-Prince trap, a CDC Wilton trap (based on attraction of ovipositing females to a black cylinder), and a specially designed sticky board against a human landing rate. They stated that for *Ae. aegypti,* these traps were not acceptable alternative to the measurement of human landing rates. The best trap, the omnidirectional Fay-Prince, caught less that 0.4 adult/h, compared with landing rates of 4.5/h.

Schoeler et al. (2004) evaluated 4 surveillance traps for *Ae. aegypti* in Iquitos, Peru (ABC-PRO; similar to the standard CDC trap with CO_2, omni-

Table 4. Mosquitoes collected by 4 different trapping devices during 21-h sampling periods in experiment 3.

Species/sex		Type of collection: BG-Sentinel + BG-Lure	MML with CO_2	MML with CO_2 + BG-lure	Fay-Prince (no CO_2, no lure)
Ae. aegypti females	Total collected	23	7	26	0
	Mean/trap/18 h[1]	2.56b	0.70a	2.60b	0.00a
	SEM	0.67	0.33	0.48	0.00
Ae. aegypti males	Total collected	7	1	8	0
	Mean/trap/18 h	0.78a	0.10a	0.80a	0.00a
	SEM	0.43	0.10	0.51	0.00
Cx. quinquefasciatus females	Total collected	2	3	2	0
	Mean/trap/18 h	0.22a	0.30a	0.20a	0.00a
	SEM	0.15	0.21	0.13	0.00
Cx. quinquefasciatus males	Total collected	0	0	0	0
	Mean/trap/18 h	0.00a	0.00a	0.00a	0.00a
	SEM	0.00	0.00	0.00	0.00
	No. of collections	9	10	10	10

[1] Mean/trap/21 h, mean number of mosquitoes caught during the 21-h sampling periods; trap days, number of days when traps worked without technical problems.

directional Fay-Prince with and without CO_2, and CDC Wilton), and compared them with the performance of a backpack aspirator and a human volunteer. None of these traps produced satisfactory results, with catching rates well below 0.1 adult *Ae. aegypti* per hour, compared with about 3 adults/h for the human volunteer. (The study did not differentiate between male and female mosquitoes.) The addition of CO_2 improved the catching rate of an omnidirectional Fray-Prince trap only to a small extent. Schoeler et al. (2004) also calculated positive indices for the sampling techniques they tested: in 85% of the 2-h sampling periods, at least 1 *Ae. aegypti* was caught by the human volunteers, in less than 5% by the Fay-Prince traps. Similar numbers were produced in our study (Fig. 8) but for 3-h sampling periods.

Russel (2004) compared CDC- and EVS-type light traps with and without the addition of CO_2, octenol, and the combination of both, against *Ae. aegypti*, *Ae. polynesiensis*, and *Cx. quinquefasciatus* in Moorea, French Polynesia. Although the addition of CO_2 had some positive effect, neither trap produced substantial catches when compared with landing collections by humans. Although biting pressure was high (a human volunteer caught between 3 and 15 *Ae. aegypti* females in 15 min), the catching rates for CO_2-baited traps were between 5 and 29 *Ae. aegypti* only during a total 72-h sampling period.

The human landing rates described in all of the aforementioned studies were higher than those described in our study (Tables 1 and 3). One of the reasons for this lower biting pressure could be the high numbers of other potential human hosts in the test area. A high number of students and staff were constantly present on the campus and near the traps, either walking around, or sitting in the vicinity. Taking into account the high competition because of these other hosts, the collection rate of the BG-Sentinel trap showed a remarkable sensitivity.

The human landing collections and the low number of trap catches with the Fay-Prince trap reported herein correspond well with a recent study in the same test area (Silva et al. 2005).

Comparison to sticky ovitraps: Ovitraps are con-

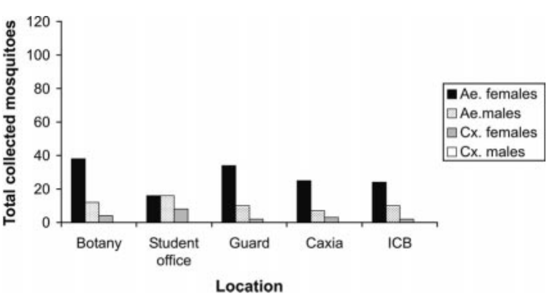

Fig. 6. Total number of mosquitoes collected at the different test locations during experiment 3. Ae., *Ae. Aegypti*; Cx., *Cx. quinquefasciatus.* Bars with the same letters are not significantly different ($P > 0.05$).

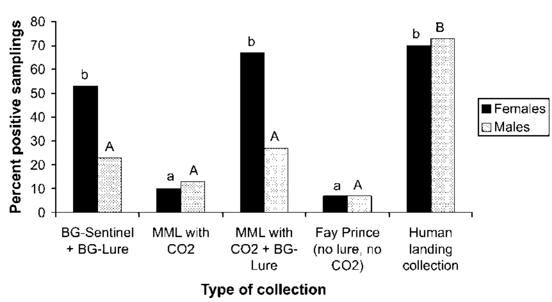

Fig. 7. Percentage of sampling periods that were positive for 1 or more *Ae. aegypti* from 3-h sampling collections from experiments 2 and 3. Bars with the same letters are not significantly different ($P > 0.05$).

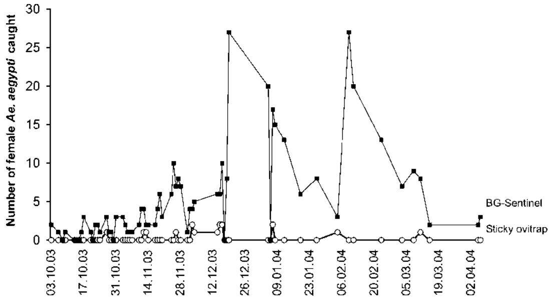

Fig. 8. Long-term comparison between the BG-Sentinel trap and a sticky ovitrap. The rainy season began at the end of November. The *y*-axis shows the mean number of female *Ae. aegypti* caught per 24 h; the *x*-axis shows the date when collections were made.

sidered as cheap and relatively sensitive tools in the surveillance of populations of *Ae. aegypti* (Focks 2003). Because of their low price and easy deployment, they can be used in large numbers. A drawback of these traps is the time-consuming processing of trap catches that is necessary to hatch eggs and rear larvae for identification. They are also inaccurate because we do not know the relationship between the number of deposited eggs and the number of females laying the eggs (Ritchie et al. 2003) or the number of host-seeking females. In addition, the number of eggs in an ovitrap and the proportion of positive ovitraps are highly influenced by the availability of competing oviposition sites in the vicinity of a trap. The 1st point is addressed by the development of sticky ovitraps that make data analysis much easier by catching the ovipositing female on a glue board. Ritchie et al. (2003) used sticky ovitraps similar to the ovitraps used in our study and compared them with conventional ovitraps in the city of Cairns in Queensland, Australia. They found no significant difference in their sensitivity for *Ae. aegypti*. They report catching rates of between 0.1 and 0.2 mosquito/day and of between less than 0.1 and 0.5 mosquito/day in a 2nd study (Ritchie et al. 2004), which corresponds well to the mean rate observed in our study (0.25).

In comparison, the BG-Sentinel trap not only showed a larger catching rate (5.25 mosquitoes/day) and a higher proportion of days with a least 1 mosquito in the trap (88% vs. 18%) but also a much broader range of mosquito numbers. The catching rate of the BG-Sentinel trap increased considerably after the beginning of the rainy season at the end of November, whereas the number of mosquitoes in the sticky ovitraps did not change (Fig. 8). One explanation could be that the rise in the mosquito population is not apparent because of increased competition for the ovitraps from additional oviposition sites created by the rains.

General considerations: From our experiments no conclusions could be drawn about the role of attractants versus trap design because the BG-Sentinel was tested only with the BG-Lure. The BG-Lure clearly had a strong enhancing effect when combined with the CO_2-generating trap, but from our experiments, it is still unclear how much the lure contributes to the trapping performance of the BG-Sentinel trap when no CO_2 is added. We assume that the addition of CO_2 may further increase the catching rate of the BG-Sentinel, but the issue remains open to what extent the efficacy could be increased.

Besides *Ae. aeygypti*, only *Cx. quinquefasciatus* was present in the study area and only in low numbers. Therefore, the results with the latter species should be interpreted with care. The number of collected *Cx. quinquefasciatus* was almost the same in the gas-powered trap and the BG-Sentinel trap. The addition of the BG-Lure to the MML trap had no significant effect. This finding may indicate that the trap design of the BG-Sentinel trap by itself is probably well suited to collect other anthropophagic species and could become an important tool for early detection of invasive mosquito species.

In all experiments with landing collections by humans, there was a remarkable difference in the collection rates of male *Ae. aegypti* by human volunteers and the collection rates by using the trapping devices. No trap caught males as efficiently as the human volunteers. Unlike females, the male mosquitoes did not really land on the exposed legs and arms, but they often had short contacts during which they could be caught by skillful test persons. It is well known that males occur around human hosts where they can intercept and mate with females seeking blood meals (Hartberg 1971). It is possible that the trap devices used in this study miss some features that attract males toward humans. An alternative explanation could be that human volunteers are more efficient at catching the males than the traps, where the insects have to be lured close to the openings of the suction fan. Compared with the CO_2 trap systems, the BG-Sentinel trap caught males in similar rates, indicating that CO_2 is probably not the crucial factor in their attraction.

Although we assume that most of the females that were caught in the BG-Sentinel were host seeking ones, this was not checked in this study. Further investigations are needed to determine the physiological state of the mosquitoes caught.

Final remarks: Our results demonstrate that the BG-Sentinel trap and the BG-Lure are highly effective tools for monitoring populations of *Ae. aegypti* under real-life conditions in urbanized areas. The BG-Sentinel trap is almost as specific as human volunteers at collecting adult female *Ae. aegypti*, even without the addition of CO_2. Therefore, this new tool could replace ethically questionable human landing collections in Dengue monitoring and control programs and could provide a new standardized artificial human sentinel. Because of its high efficiency and simplicity, this trap also could be used in control campaigns to reduce human biting rates and to suppress local mosquito populations. Further studies in other regions have to be conducted to calibrate this instrument and to check whether populations of *Ae. aegypti* in other regions respond in a similar manner to the population in Belo Horizonte.

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