The impact of CO_2 on collection of *Aedes aegypti* (Linnaeus) and *Culex quinquefasciatus* Say by BG-Sentinel® traps in Manaus, Brazil

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Carbon dioxide (CO_2) is an important component for activating and attracting host-seeking mosquitoes. The BG-Sentinel® trap is a well-established monitoring tool for capturing Culicidae, but CO_2 role for the trap effectiveness has not been evaluated in highly urbanised areas. The objective was to evaluate the effectiveness of BG-Sentinel traps baited with and without CO_2 for capturing urban mosquitoes. Fifteen areas were selected within the city of Manaus, Brazil, where four BG-Sentinels were operated for 24 h, two of them with CO_2 and two without CO_2. Captured Aedes aegypti *females were dissected for the determination of their parity status. A significantly higher proportion of traps (from 32-79%) were positive for female* Ae. aegypti *when using the BG-Sentinel with CO_2 (χ^2 = 11.0271, p < 0.001). Catches of female* Culex *spp were six times higher in CO_2 traps (Mann-Whitney* U *test = 190.5; p = 0.001). Parity rates were similar for both traps. This study showed that CO_2 has primarily an enhancing effect on the efficacy of BG-Sentinel for capturing* Culex *spp in Manaus. For* Ae. aegypti*, the positivity rate of the trap was increased, when CO_2 was added.*

Key words: adult mosquitoes - dry ice - urban area

Detection of chemical cues emitted by vertebrate hosts is important for the host-finding behaviour of mosquitoes. Carbon dioxide (CO_2) is a known attractant for mosquitoes and fluctuations in the atmospheric concentration of the gas can indicate the presence of a host (Reeves 1990, Dekker et al. 2005). CO_2 also activates the host-seeking behaviour of mosquitoes, including *Aedes aegypti* (L.) (Eiras & Jepson 1991). CO_2-baited traps have been widely used to increase catch rates of mosquitoes, including for monitoring of *Ae. aegypti* with Centers for Disease Control (CDC) traps in the United States of America (Service 1992, Canyon & Hii 1997).

In Brazil, the National Dengue Control Program recommends monitoring of *Ae. aegypti* based on larval surveys (MS/FUNASA 2002). Nevertheless, traps for capturing adult mosquitoes such as the MosquiTRAP® (Gama et al. 2007) and the BG-Sentinel® (Kröckel et al. 2006) have been evaluated as new monitoring technologies for dengue vectors in Brazil.

The Biogents-Sentinel™ trap (BGS) (Biogents AG, Regensburg, Germany) attracts mosquitoes by visual cues, by the imitation of convection currents of human beings and by olfactory baits which are released through a dispenser [BG-Lure (BGL)] which is placed inside of the trap (Kröckel et al. 2006). The BGL contains substances that are found on human skin, such as ammonia, lactic acid and caproic acid that also attracts for host seeking females *Ae. aegypti* (Geier et al. 1999, Bosch et al. 2000).

The BGS has been used for capturing Culicidae, especially *Ae. aegypti, Aedes albopictus and Culex* spp (Williams et al. 2006, 2007), including studies with parity rates (Maciel-de-Freitas et al. 2007) and detection of new infestations with mosquitoes (Ritchie et al. 2006). In these studies, BGS traps were used without addition of CO_2 which is cost and labour-intensive, as dry ice is not available everywhere and CO_2 cylinders are heavy to carry. Other suction traps for mosquitoes, such as CDC traps, are routinely used with CO_2 in order to obtain sufficient collections (McNelly 1989). In direct trap comparisons it was shown that BGS traps without CO_2 capture significantly more female *Ae. aegypti* than CO_2-baited encephalitis vector surveillance traps (Williams et al. 2006) and significantly more female *Ae. albopictus* than CO_2-baited CDC traps (Meeraus et al. 2008), but the effect of CO_2 on catch rates of BGS traps for urban mosquitoes, mainly *Ae. aegypti* and *Culex* spp, has not been investigated in Brazil.

The objective of the present paper is to compare the effectiveness of BGS traps for capturing urban Culicidae, such as *Ae. aegypti* and *Culex quinquefasciatus* when used with and without CO_2 in an urban area in Brazil. Additionally, the physiological state of female *Ae. aegypti* was determined.

The study was conducted in 15 urban areas within the neighbourhood Cidade Nova, in the northern region of the city of Manaus, state of Amazonas, Brazil (3°6′0″S 60°1′0″W). Cidade Nova is the most populated neighbourhood of Manaus, with more than 300,000

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inhabitants, and was chosen as a study site because of high larvae indices and regular sanitation in most of the houses. All 15 areas were at least 250 m apart from each other and included four-seven quarters with a total of approximately 120 houses.

The BGS traps were supplied with approximately 3 kg of pulverised dry ice (CARBOMAN Ltda, Manaus). Bottles of 5 L PET were adapted for being used as recipients for the dry ice. A hole of 4 mm was drilled in the lid of the bottle and a polyethylene tube of 4 mm diameter and 1 m of length was pulled through. The connection of the tube and the lid was closed air-tight with the help of hot glue. The bottle was isolated with a double layer of bubble foil and placed inside of a 20 L-Styrofoam box. The polyethylene tube was connected to a Biogents-CO_2-nozzle and this nozzle was attached to the end of a mounting pole of the trap (bg-sentinel.com/bilder/BG-Sentinel_Manual_Addition_of_CO2.pdf) (Figure).

In each of the 15 areas, four houses were randomly chosen (flipping of a coin) to receive BGS traps with BGL. Two of the four traps per area were additionally baited with CO_2. The traps were installed in the peridomestic area of houses, sheltered from sunlight and rain. All traps were installed in the morning and operated for a 24 h period. Catch bags were identified and sent to the Entomology Department of the Tropical Medicine Foundation of Amazonas (FMT-AM) in Manaus. Three of the 60 traps were excluded from the trial, because inhabitants were absent at the end of the trapping period, or the traps were turned off. Trap catches were performed in January 2009.

A: filled PET-bottle with 3 Kg of dry ice and carbon dioxide (CO_2)-nozzle, surrounded by bubble foil; B: sealed 20L-Styrofoam box with CO_2-nozzle; C: BG-Sentinel® trap with CO_2-nozzle (arrow) which is connected by a plastic tube to the dry ice-filled PET-bottle.

Captured mosquitoes were counted and sexed under a stereomicroscope. *Aedes* were identified to species and other Culicidae were identified to genus with the help of a dichotomic identification key (Consoli & Lourenço-de-Oliveira 1994). Female individuals of *Ae. aegypti* were dissected and the parity status (parous, nulliparous) was evaluated for females in egg development stage ≤ Christopher's stage II (Detinova 1962, Reiter & Nathan 2001). Females in Christopher's stage >II were documented as "late ovarian development stages".

For *Ae. aegypti* and *Culex* mosquitoes, statistical differences between the catches of BGS traps with and without CO_2 were assessed using the nonparametric Mann-Whitney *U* test. For *Ae. aegypti*, the chi-square test was used to investigate if the proportions of positive catch rates differ between the two trap types. Parity rates were compared by Fisher's exact test. Due to the low *Ae. albopictus* catch rates, comparisons between traps were not statistically evaluated. Statistical analysis was performed using the statistical software R 2.12.2 (r-project.org) (The R Foundation for Statistical Computing, 2010). The Ethical Research Committee (CEP) of studies involving human beings from the FMT-AM approved this project (approbation 1906 - registration CEP 1024-08).

BGS traps (n = 57) collected 2,924 Culicidae, where 2,699 (92.3%) belonged to the genus *Culex* and 225 (7.7%) to the genus *Aedes*. Of the 225 *Aedes* mosquitoes, 197 (88%) were identified as *Ae. aegypti* and 28 (12%) as *Ae. albopictus*. BGS traps baited with BGL and CO_2 (n = 29) captured significantly higher mean numbers of female (Mann-Whitney, $p = 0.03$) and sum of male and female *Ae. aegypti* (Mann-Whitney, $p = 0.04$), than traps baited with BGL only (n = 28), but no significant difference was observed for males (Mann-Whitney, $p = 0.07$) (Table I). For BGS traps without CO_2, nine out of 28 traps (32%) were positive for the presence of female *Ae. aegypti* and for traps with CO_2, 23 out of 29 traps (79%) were positive ($\chi^2 = 11.0271$, $p < 0.001$). Interestingly, BGS traps without CO_2 collected the highest maximum number of female and male *Ae. aegypti* per 24 h trapping period.

Traps with CO_2 captured six times more female and almost four times more male *Culex* spp, than traps without CO_2. Significant difference between two different trap configurations was only observed for females (Mann-Whitney *U* test: *Culex* females: $U = 190.5$; $p = 0.001$; *Culex* males: $U = 302$; $p = 0.095$) (Table I).

Addition of CO_2 slightly increased the catch rates of *Ae. aegypti* females and males (by 23% and 9%, respectively). The better performance of the traps with CO_2 is more pronounced in the comparison of the proportions of positive trapping periods, which was significantly higher, when CO_2 was used.

Many trap types are routinely used with CO_2 in order to obtain sufficient trapping efficacies. The CDC trap was shown to catch significantly higher numbers of *Ae. aegypti* in French Polynesia when it was used with CO_2 (Russel 2004).

Although the BGS trap was especially developed for capturing *Ae. aegypti*, catches of *Culex* mosquitoes have been reported (Kröckel et al. 2006, Williams et al. 2006). This might be due to the BGS trap's imitation

of human odour plumes. In the present study, six times higher number of female *Culex* spp were captured by the traps that were operated with CO_2. This suggests that this kairomone might be an important attractant for this mosquito genus. Similar results were described before for the CDC trap (Russel 2004). The high catch rates of up to 272 *Culex* females with CO_2 and up to 57 *Culex* females without CO_2 (mainly *Cx. quinquefasciatus*, personal observation of TMF de Ázara) per 24 h shows that the BGS trap might be a useful tool for the monitoring of diseases that are transmitted by the species in urban areas in Brazil, like Oropouche fever or Bancroftian Filariosis. The high catch rates of *Culex* mosquitoes in both BGS configurations demonstrate that these mosquitoes are predominant in our study area. This information was confirmed by the Foundation of Health Vigilance in Manaus (L Mustafa, unpublished observations).

It is interesting to note that the positive effect of CO_2 on the collection rate of the traps is most pronounced in mosquito species that occur in high densities, less pronounced in mosquito species that occur in low densities. It might be that in urbanised areas with a high density of human hosts and a high background level of atmospheric CO_2, the CO_2 signals from the trap attract mosquitoes only over a short-range distance.

These results may have a practical importance for monitoring programs in urban areas. For instance, for monitoring *Ae. aegypti* with BGS traps in urban areas, CO_2 is not necessarily required, which minimizes costs and labour. Even if positivity of the BGS trap for *Ae. aegypti* females was higher when CO_2 was used, the high costs and operational labour of using CO_2 from cylinders or dry ice might not to be worth it. Instead, the number of traps could be increased in order to capture a sufficient number of individuals. As conditions can vary considerably between different geographic areas, we suggest evaluating the traps performance with and without CO_2, before a bigger experiment with BGS traps is being started. For researchers who work with *Culex* however, it is highly recommendable to add CO_2, if very high catch rates are required.

Dissections were performed with 104 of the 105 captured *Ae. aegypti* females. The traps with and without CO_2 captured 59 and 45 females, respectively (Table II). The presence of fresh blood was detected in three out of the 27 females in early ovarian development stages that were captured by traps with CO_2 and in eight out of the 17 females in early ovarian development stages that were captured by the traps without CO_2 (Table II). The parous rate [parous/(nulliparous + parous)] of *Ae. aegypti* was 92.6% and 82.4% for traps with and without CO_2, respectively and 88.6% for all traps together. The proportions of parous and nulliparous females did not differ significantly between the two trap types (Fisher's exact test: $p = 0.359$).

Theoretically, the BGS with BGL should be especially attractive for host-seeking female mosquitoes. Maciel-de-Freitas et al. (2006) found the highest percentage of recaptured individuals in to be in initial stages of ovarian development, what reflects that these females were host seeking. In contrast to this, we found almost 60% of *Ae. aegypti* females collected to be in ovarian development stages > II, what means that this females have taken a blood meal recently and are developing a batch of eggs. Morais (2009) found similar results in field studies conducted in the urban area of Belo Horizonte, Brazil, where 71% of female *Ae. aegypti* captured with BGS traps were gravid. As *Ae. aegypti* is known to take more than one blood meal during a single gonotrophic cycle (Barata et

TABLE I

Aedes aegypti and *Culex* spp [mean ± standard error (SE)] in BG-Sentinel traps (BGS) baited with BG-Lure (BGL) and with or without carbon dioxide (CO_2) in Manaus, state of Amazonas, Brazil

		BGS + BG L + CO_2		BGS + BGL
Ae. aegypti				
Female	Mean	2.0	-	1.6
	SE	0.39	-	0.81
	p	-	0.038[a]	-
	Sum	59	-	46
Male	Mean	1.6	-	1.5
	SE	0.43	-	0.94
	p	-	0.07	-
	Sum	49	-	43
Culex spp				
Female	Mean	42.7	-	7.3
	SE	12.23	-	2.26
	p	-	0.001[a]	-
	Sum	1282	-	205
Male	Mean	32.0	-	9.0
	SE	12.06	-	2.38
	p	-	0.095	-
	Sum	961	-	251

a: statistical difference (Mann-Whitney *U* test) between catches of female for the same specie and male also for the same specie of the two different trap configurations.

TABLE II

Physiological status of female *Aedes aegypti* captured with BG-Sentinel traps (BGS) baited with BG-Lure (BGL) and with or without carbon dioxide (CO_2) in Manaus, state of Amazonas, Brazil

	Ae. aegypti		
	BGS + BGL + CO_2 n (%)	BGS + BGL n (%)	Total n (%)
Nulliparous	2 (3.4)	3 (6.7)	5 (4.8)
Parous	25 (42.4)	14 (31.1)	39 (37.5)
> stage III	32 (54.2)	28 (62.2)	60 (57.7)
Total	59 (100)	45 (100)	104 (100)
Engorged	3 (0.5)	8 (18)	11 (10.6)

al. 2001), our observation could indicate that some of the females were seeking for a host or that the BGS traps are not only attractive for host-seeking mosquitoes. The visual cue of the black funnel for example could be attractive to gravid females looking for oviposition sites. The observation that 88.6% of the *Ae. aegypti* females were parous could reflect high survival rates in Manaus and thus a high transmission risk of dengue viruses.

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