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Effect of CO_2 and 1-octen-3-ol attractants for estimating species richness and the abundance of diurnal mosquitoes in the southeastern Atlantic forest, Brazil

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Studies have shown that both carbon dioxide (CO_2) and octenol (1-octen-3-ol) are effective attractants for mosquitoes. The objective of the present study was to evaluate the attractiveness of 1-octen-3-ol and CO_2 for diurnal mosquitoes in the southeastern Atlantic forest. A Latin square experimental design was employed with four treatments: CDC-light trap (CDC-LT), CDC-LT and 1-octen-3-ol, CDC-LT and CO_2 and CDC-LT with 1-octen-3-ol and CO_2. Results demonstrated that both CDC-CO_2 and CDC-CO_2-1-octen-3-ol captured a greater number of mosquito species and specimens compared to CDC-1-octen-3-ol; CDC-LT was used as the control. Interestingly, Anopheles (Kerteszia) *sp. was generally attracted to 1-octen-3-ol, whereas* Aedes serratus *was the most abundant species in all Latin square collections. This species was recently shown to be competent to transmit the yellow fever virus and may therefore play a role as a disease vector in rural areas of Brazil.*

Key words: insect attractants - disease vectors - mosquito control

Carbon dioxide (CO_2) is an effective attractant for collecting mosquitoes (Diptera, Culicidae) and thus for monitoring vector-borne diseases (Reeves 1951, Silver 2008). Octenol (1-octen-3-ol) was first demonstrated to be a mosquito attractant by Takken and Kline (1989). These authors successfully utilised both 1-octen-3-ol and CO_2 to attract *Aedes taeniorhynchus* (Wiedemann) in Everglades National Park, Florida, USA. Later Kline et al. (1990) compared mosquito attraction to 1-octen-3-ol and CO_2 in the Everglades and verified that few mosquito species responded in large numbers to 1-octen-3-ol alone. However, there was an increase in the amount of mosquito species due to the synergistic effect between CO_2 and 1-octen-3-ol. Interestingly, *Culex* sp. showed poor response to 1-octen-3-ol alone or in combination with carbon dioxide. Taken together, Kline et al. (1990) demonstrated that the synergistic effect of both chemicals attracted zoophilic species, whereas the attraction for ornithophilic species, such as *Culex* sp. was poor.

In South America, CO_2 was successfully used for collecting mosquitoes in the Atlantic forest (Forattini et al. 1993a). Nocturnal collections were performed with CDC-CO_2 traps in an irrigated rice field in the Vale do Ribeira, state of São Paulo (SP), Brazil. *Aedes scapularis* (Rondani), *Anopheles albitarsis* Lynch Arribálzaga, *Mansonia indubitans* Dyar and Shannon and *Coquillettidia venezuelensis* (Theobald) were abundant. These results may be explained by the adaptation of mosquitoes to the modified environment and the high concentration of CO_2 used. Although Forattini et al. (1993a) successfully utilized CO_2, this attractant was not yet applied in other circumstances, i.e., diurnal catches with a lower flow rate on a forested site. Reisen et al. (2000) reported that CO_2 is released at an average rate of 500 mL/min for a 1.5-kg block of dry ice in a similar trap. According to Reeves (1953), a high flow rate of CO_2 (> 500 mL/min) attracts mosquito species that usually feed on large mammals. Therefore, a CO_2 flow rate of 250 mL/min is expected to predominately attract anthropophilic species.

Yellow fever virus (YFV) can be transmitted by mosquitoes of the genera *Haemagogus* and *Sabethes* (Vasconcelos 2003), which are common in the Atlantic forest (Forattini 1965). As YFV circulates in SP (de Souza et al. 2010) and may be carried by other vectors (Cardoso et al. 2010), it is important to establish which mosquito species are involved in the dynamics of transmission. Consequently, vector surveillance should employ efficient collection methods that allow for definitive species identification while also providing an accurate representation of species abundance and richness.

Therefore, the objectives of this study were the following: (i) to evaluate a CO_2 collection method in remote areas, (ii) to compare the attractiveness of 1-octen-3-ol alone, CO_2 alone, or CO_2 plus 1-octen-3-ol with a standard CDC-light trap (CDC-LT) for diurnal mosquitoes and (iii) to discuss the possible implementation of these methods for the surveillance of YFV vectors in the southeastern Atlantic forest.

MATERIALS AND METHODS

Study design - A field trial was carried out in a remnant of the Atlantic forest; the selected site was a dense ombrophilous forest in a mountainous landscape (Fig-

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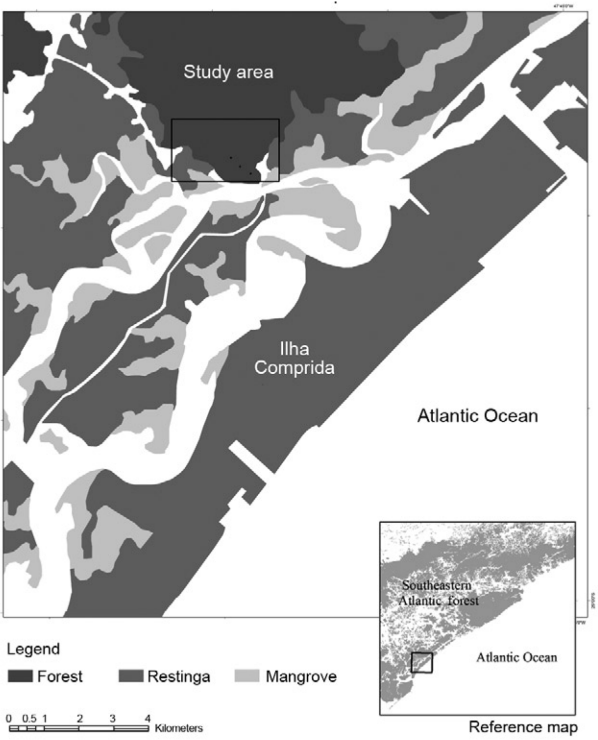
ure). Three collection sites, located 200 m apart, were chosen in Sítio Itapuã, Cananéia, SP, Brazil. Latin square analyses, consisting of one CDC-LT, one CDC-LT with 1-octen-3-ol, one CDC-LT with CO_2 and one CDC-LT with CO_2 plus 1-octen-3-ol were performed at each site.

Mosquito collections were carried out over a 12 h period, from 06:00 am-06:00 pm. Collections were performed in site 1, in the days 8, 10, 17 and 19, in site 2, in the days 24, 26 November, 1, 3 December, and in site 3, in the days 8, 10, 15 and 17 December 2009. Each treatment was tested in all possible positions at each collection site. Previous studies have determined that *Aedes* sp. were attracted at distances of 3 and 7 m from the CO_2 source (McIver & McElligott 1989) and that CDC-LTs placed 15 m apart act as independent traps (Brown et al. 2008). Therefore, to avoid interference among traps, they were placed approximately 16 m apart.

Species identifications were based on the morphological identification keys proposed by Lane (1953), Correa and Ramalho (1956) and Forattini (2002).

Traps - The CDC-LTs employed in this study were identical to those used by Forattini et al. (1993a) with one exception; the four large-sized batteries were replaced by a rechargeable battery, which supplied electric power for the 3 W incandescent bulb and the 6 V engine. The 1-octen-3-ol mosquito attractant (Koolatron™), an alcohol, consisted of a solid strip containing 3.72 g of 1-octen-3-ol. CO_2 was supplied from a 4.5 kg cylinder (White Martins™). The scheme proposed by Addison et al. (1979) was employed with the following modifications. First, a low-flow valve (Swagelok™) was connected to the cylinder allowing CO_2 release to be adjusted to simulate a human host (250 mL CO_2 per minute) (Reeves 1953). Also, a tube (Swagelok™) 6.25 cm in diameter and 100 cm in length was attached to this valve. This release tube was then connected to the head of the CDC-LT, which was suspended at a height of 1.5 m. All other CDC-light traps were also suspended at a height of 1.5 m.



Sítio Itapuã, Cananéia, Vale do Ribeira (47º50”20W 24º51”48S, South American Datum 69); reference map: the southeastern Atlantic forest (source: National Institute for Space Research).

Species richness - Species richness in each trap was divided by total species richness (Table I) to estimate a third variable, which was employed as the sensibility of the collection method. To compare sensibility among treatments, a multi-model framework was established. Models were as follows: model 1a (neutral model), all treatments were considered to have identical sensibility; model 2a (multiple effects), each treatment has a different sensibility; model 3a (control effect), treatment 1 (control) has a different sensibility, but others have equal sensibility; model 4a (CO_2 effect), treatments 1 and 2 have equal sensibility, which differs from those of treatments 3 and 4; model 5a (1-octen-3-ol effect), treatments 1 and 3 have identical sensibility, which differs from those of treatments 2 and 4; model 6a (synergistic effect) treatment 4 has a different sensibility, but others have equal sensibility.

Abundance - Total abundance was the sum of number of specimens collected using a specific treatment. This variable was considered to have a log-normal distribution. Total abundance was compared among treatments using CDC-LT as a control, and a second multi-model framework was applied. Models were as follows: model 1b (neutral model), all treatments were considered to have identical abundance; model 2b (multiple effects), each treatment has a different abundance; model 3b (control effect), treatment 1 (control) has a different abundance, but others have equal abundance; model 4b (CO_2 effect), treatments 1 and 2 have equal abundance, which differs from those of treatments 3 and 4; model 5b (1-octen-3-ol effect), treatments 1 and 3 have identical abundance, which differs from those of treatments 2 and 4; model 6b (synergistic effect) treatment 4 has a different abundance, but others have equal abundance.

Exploratory analysis - Shannon-Wiener and Simpson-Yule diversity scores were calculated for each treatment at each collection site. Analysis of variance was performed in order to compare each of these alpha diversity scores among sites of collection.

Model selection analysis - All analyses were conducted with the software package R 2.9.2 (http://www.r-project.org.br/). The Akaike Information Criteria (AIC) was applied to differentiate distances among models in each multi-model framework. AIC values were relative distances among observed models from the real or expected model, which was based on the available data. Moreover, the AIC value was used as a measure of plausibility for each model. If the difference between two AIC values was more than 2, the model with a lower AIC value was considered to be more plausible than the others. One advantage of applying AIC to evaluate collection methods is the use of many concurrent models that might explain observed differences. Here, six concurrent models, 1a-6a and 1b-6b, were each evaluated for richness and abundance among treatments. In addition, model selection was applied for each collection site. Further information about this approach has been published previously (Hobbs & Hilborn 2006).

RESULTS

Collections generated 48 sample units, representing 576 h of effort. As no male mosquitoes were collected, all results represent female mosquitoes. A total of 3,507 mosquitoes belonging to 33 species were collected (Table I). No differences in individual alpha diversity scores were observed among collection sites (Table II). Additionally, no difference in model selection was observed among sites of collection (Supplementary data). Therefore, model selection analysis was performed with richness and abundance data pooled from all collection sites.

TABLE I

Species, type of treatment (T) and number of females, Atlantic forest, 2009

Species	T1[a]	T2[b]	T3[c]	T4[d]	Total
Aedes (*Howardina*) *fulvithorax*	0	1	0	1	2
Aedes (*Ochlerotatus*) *hastatus*	0	0	8	7	15
Aedes (*Ochlerotatus*) *scapularis*	0	1	8	9	18
Aedes (*Ochlerotatus*) *serratus*	7	16	368	308	699
Aedes (*Protomacleaya*) *terrens*	0	1	7	2	10
Anopheles (*Anopheles*) *mediopunctatus*	0	0	0	1	1
Anopheles (*Kerteszia*) *bellator*	0	3	30	60	93
Anopheles (*Kerteszia*) *cruzii*	0	0	24	74	98
Anopheles (*Kerteszia*) *homunculus*	0	0	8	25	33
Coquillettidia (*Rhynchotaenia*) *chrysonotum*	1	4	17	42	64
Culex (*Culex*) *bidens*	0	0	6	4	10
Culex (*Melanoconion*) *sacchettae*	0	0	22	7	29
Limatus durhamii	23	8	200	198	429
Limatus flavisetosus	4	1	459	215	679
Onirion personatum	0	0	1	0	1
Psorophora (*Janthinosoma*) *albigenu*	0	2	1	7	10
Psorophora (*Janthinosoma*) *ferox*	2	15	45	47	109
Runchomyia (*Runchomyia*) *cerqueirai*	0	0	1	0	1
Runchomyia (*Runchomyia*) *frontosa*	0	0	15	9	24
Runchomyia (*Runchomyia*) *reversa*	0	0	19	5	24
Runchomyia (*Runchomyia*) *theobaldi*	1	0	8	4	13
Sabethes (*Peytonulus*) *soperi*	0	0	5	2	7
Sabethes (*Sabethinus*) *intermedius*	0	0	2	0	2
Trichoprosopon pallidiventer	0	1	23	8	32
Trichoprosopon townsendi	0	0	1	0	1
Wyeomyia aporonoma	0	0	159	102	261
Wyeomyia occulta	0	0	31	24	55
Wyeomyia (*Phoniomyia*) *pallidoventer*	0	0	24	18	42
Wyeomyia (*Phoniomyia*) *quasilongirostris*	0	0	15	4	19
Wyeomyia (*Phoniomyia*) *theobaldi*	0	0	0	4	4
Wyeomyia (*Prosopolepis*) *confusa*	24	10	279	169	482
Wyeomyia shannoni	0	0	39	11	50
Wyeomyia (*Wyeomyia*) *pertinans*	0	0	124	66	190
Total abundance	62	63	1,949	1,433	3,507
Total species richness	7	12	30	29	33

a: CDC-light trap; *b*: CDC-octenol (1-octen-3-ol); *c*: CDC-carbon dioxide (CO_2); *d*: CDC-CO_2-1-octen-3-ol.

TABLE II

Analysis of variance of alpha diversity scores

Treatments	Shannon-Wiener	Simpson-Yule
CDC-LT[a]	1.12	0.62
CDC-1-octen-3-ol[a]	1.68	0.76
CDC-CO$_2$[a]	2.12	0.82
CDC-CO$_2$-1-octen-3-ol[a]	2.16	0.82
CDC-LT[b]	1.08	0.62
CDC-1-octen-3-ol[b]	1.55	0.78
CDC-CO$_2$[b]	2.37	0.87
CDC-CO$_2$-1-octen-3-ol[b]	2.39	0.87
CDC-LT[c]	1.12	0.61
CDC-1-octen-3-ol[c]	1.84	0.83
CDC-CO$_2$[c]	2.33	0.86
CDC-CO$_2$-1-octen-3-ol[c]	2.57	0.90
ANOVA one-way	F = 0.11 ($p < 0.89$)	F = 0.14 ($p < 0.87$)

a: treatment applied in collection site 1; *b*: treatment applied in collection site 2; *c*: treatment applied in collection site 3; CDC-LT: CDC-light trap; CO$_2$: carbon dioxide; 1-octen-3-ol: octenol.

Species richness - Collected species predominately belonged to the Sabethini and Aedini tribes. The species richness was seven for CDC-LT, 12 for CDC-1-octen-3-ol, 30 for CDC-CO$_2$ and 29 for CDC-CO$_2$-1-octen-3-ol, and sensibility was 21%, 36%, 91% and 88%, respectively. In the first multi-model framework, models 1a (AIC = 72), 2a (AIC = 30), 3a (AIC = 26), 4a (AIC = 32), 5a (AIC = 56) and 6a (AIC = 59) showed great divergence. The most plausible model was 3a (control effect), which was followed by the equally plausible models 2a (multiple effects) and 4a (CO$_2$ effect). Models 1a (neutral model), 5a (1-octen-3-ol effect) and 6a (synergistic effect) were not plausible.

Abundance - Mosquito abundance varied among treatments (Table I). High relative abundance of *Aedes serratus* (Theobald) (20%), *Limatus flavisetosus* Oliveira Castro (19%), *Wyeomyia confusa* (Lutz) (14.5%) and *Limatus durhami* Theobald (12%) was observed. Numbers of *Ae. serratus*, *Li. flavisetosus*, and *Wy. confusa* were highest in treatment 3 (368, 459 and 482, respectively). The log-natural means and the standard deviations of abundance among the three collection sites were 2.95 ± 0.5 (CDC-LT), 2.75 ± 0.92 (CDC-1-octen-3-ol), 6.43 ± 0.36 (CDC-CO$_2$) and 6.15 ± 0.17 (CDC-CO$_2$-1-octen-3-ol). Results of the second multi-model framework were as follows: models 1b (AIC = 673), 2b (AIC = 20), 3b (AIC = 12), 4b (AIC = 44), 5b (AIC = 235) and 6b (AIC = 235). The most plausible model was 3b (control effect), which was followed by models 2b (multiple effects) and 4b (CO$_2$ effect). Models 1b (neutral model), 5b (1-octen-3-ol effect) and 6b (synergistic effect) were not plausible. Although total abundance was not correlated to 1-octen-3-ol presence, *Anopheles* (*Kerteszia*) sp. responded well to this attractant. For example, CDC-CO$_2$-1-octen-3-ol collected three times more *Anopheles cruzii* Dyar and Knab and two times more *Anopheles bellator* Dyar and Knab compared to CDC-CO$_2$ (Table I). Further, while 25 specimens of *Anopheles homunculus* Komp were collected using CDC-CO$_2$-1-octen-3-ol, none were collected in the CDC-LT.

DISCUSSION

First and second multi-model frameworks showed similar results. Neutral models 1a (AIC = 72) and 1b (AIC = 673) were the least plausible. Thus, the results of sensibility and abundance among treatments were not merely caused by random effects. Models 3a (AIC = 26) and 3b (AIC = 12) of the control effect were very plausible and showed that the sensibility and abundance of the CDC-LT were different from other traps. The CDC-LT was an efficient control and the attractants 1-octen-3-ol and CO$_2$ influenced the capture of mosquitoes. Models 2a (AIC = 30) and 4a (AIC = 32) were equally plausible, indicating that each treatment had a different sensibility and that the contribution of CO$_2$ was strong. Models 2b (AIC = 20) and 4b (AIC = 44) suggested that abundance was different for each treatment and that there was a high CO$_2$ effect. The CDC-CO$_2$ trap had the highest species richness (30), sensibility (91%) and total abundance (1,949). There was not a relevant 1-octen-3-ol effect (models 5a, AIC = 56 and 5b, AIC = 235) or synergistic effect (models 6a, AIC = 59 and 6b, AIC = 235). However, the nocturnal mosquitoes *An. cruzii* and *An. bellator*, which are *Plasmodium* sp. vectors (Marrelli et al. 2007), were caught at higher frequencies in the CDC-CO$_2$-1-octen-3-ol trap. Although, Rubio-Palis (1996) stated that 1-octen-3-ol is best omitted as an attractant when working with *Anopheles albimanus* Wiedemann and *Anopheles aquasalis* Curry, the overall ineffective contribution of 1-octen-3-ol to the collection of diurnal mosquitoes was not expected.

In our study, the 1-octen-3-ol emission rate was approximately 0.05 mg per hour. This emission rate is equivalent to that of an ox (0.025-0.043 mg/h) (Clements 1999). Kline et al. (1990) successfully collected mosquitoes using 3 mg/h of 1-octen-3-ol to simulate the amount released by 60 oxen. In addition, Becker et al. (1995) employed an even greater amount of 1-octen-3-ol (13 mg/h) in their studies. In this work, despite the high concentrations of 1-octen-3-ol used, CDC-1-octen-3-ol performed poorly when compared to CDC-CO$_2$. In conclusion, both small amounts (0.05 mg/h) and high quantities (13 mg/h) of 1-octen-3-ol appear to be inadequate to attract mosquitoes.

Carestia and Savage (1967) compared the CDC-LT to the CDC-CO$_2$ trap and, employing the total amount of mosquito species collected, found the sensibility of the CDC-LT to be 45%. However, in the present study, the sensibility of the CDC-LT (21%) and of CDC-1-octen-3-ol (36%) was less than the 45% obtained by Carestia and Savage (1967). Additionally, the 91% sensibility of CDC-CO$_2$ and 88% of CDC-CO$_2$-1-octen-3-ol were higher than the expected 45%. As listed in Table I, CDC-CO$_2$ and CDC-CO$_2$-1-octen-3-ol shared 26 species of 33 species captured; *Aedes fulvithorax* (Lutz), *Anopheles mediopunctatus* (Lutz), *Onirion personatum* (Lutz), *Runchomyia cerqueirai* Stone, *Sabethes intermedius* (Lutz), *Trichoprosopon townsendi* Stone and *Wyeomyia theobaldi* (Lane and Cerqueira) were not shared by the CDC-CO_2 and CDC-CO_2-1-octen-3-ol traps. Consequently, it is plausible to hypothesize that the sensibility of CDC-CO_2-1-octen-3-ol had a stronger contribution of CO_2 than the synergistic effect of CO_2 plus 1-octen-3-ol.

The synergistic effect is based on the neural excitement of mosquitoes stimulated by CO_2 and 1-octen-3-ol. Maxillary palps of mosquitoes contain sensory neurons that respond to small changes in CO_2 concentration in the air. Additionally, 1-octen-3-ol sensitive neurons are present on the antennae of females (Clements 1999). Stimulation of both CO_2-palp and 1-octen-3-ol antennae can increase mosquito response to attractants. Low emission rates of 1-octen-3-ol (0.05 mg/h) might avoid the synergism with CO_2. Although Kline et al. (1990) observed a synergistic effect with mosquito species in Florida, Becker et al. (1995) did not find CO_2 synergism in Germany. These authors collected more specimens of *Aedes vexans* (Meigen) and *Aedes rossicus* Dolbeski, Gorickaja and Mitrofanova when using a CDC-CO_2 trap compared to the CDC-CO_2-1-octen-3-ol trap. In addition, the synergistic effect of CO_2 (dry ice 100 g/h) and 1-octen-3-ol (0.092 ± 0.015 mg/h) was significantly different from human attraction for collecting *An. albimanus* and *An. aquasalis* (Rubio-Palis 1996).

The release rate of 1-octen-3-ol must be investigated further before its synergistic effect with CO_2 can be used in traps for monitoring diurnal mosquitoes in the southeastern Atlantic forest. Few *Sabethes* specimens and no *Haemagogus* mosquitoes were collected in the entire study (Table I). Further, in the present study area, Ueno (2000) collected *Wyeomyia* sp., *Aedes crinifer* (Theobald) and *Psorophora albipes* (Theobald) mosquitoes using a Shannon trap; these species were not collected in this study. Nevertheless, CDC-CO_2 was an effective attractant for other diurnal mosquitoes. The flow of CO_2 at 250 mL/min was equivalent to the amount released by a human-sized animal placed nearby the CDC trap (Reeves 1953).

The most abundant species collected in the CDC-CO_2 traps, *Ae. serratus*, feeds on humans inside the forest (Forattini et al. 1989). YFV was isolated from *Ae. serratus* specimens collected in the state of Rio Grande do Sul in 2008 (Cardoso et al. 2010). If YFV is introduced into the Atlantic forest, then *Ae. serratus* could act as a vector. Moreover, this pan-tropical mosquito species (Forattini 2002) adapts well to rural areas (Forattini et al. 1993b) and is already a vector for YFV in human-modified environments.

Some mosquitoes collected by Forattini et al. (1993a) were either not present or collected in low frequencies in the present study (e.g., *Ae. scapularis*). This can be explained by the nocturnal feeding activity of *An. albitarsis*, *Ma. indubitans*, and *Cq. venezuelensis* and the association of these species and *Ae. scapularis* with human-modified environments. However, the diurnal sylvatic mosquitoes *Li. durhami*, *Li. flavisetosus* and *Wy. confusa* were frequently collected in the CDC-CO_2 traps. These species are associated with the abundant phytotelmata in the Atlantic forest (Forattini 1965). These species (*Li. durhami*, *Li. flavisetosus* and *Wy. confusa*) along with *Sabethes* sp., which can act as vectors of YFV (Vasconcelos 2003), share similar bionomics characteristics; for instance, they are both diurnal sylvatic species that are abundant in Atlantic forest. In addition, CDC-CO_2 seems to be a very efficient method for monitoring the abundance of these species (*Li. durhami*, *Li. flavisetosus* and *Wy. confusa*) as well as *Ae. serratus*. Moreover, the method described herein can be used in remote areas where dry ice is usually unavailable.

Other studies have compared CDC-CO_2 with Mosquito Magnet™. In relation to total abundance, Mosquito Magnet outperformed CDC-CO_2 (Johansen et al. 2003, Xue et al. 2008). However, the sensibility of CDC-CO_2 (19/27) was very close to that of Mosquito Magnet (20/27) in the study by Johansen et al. (2003). Moreover, in another study, 13 species were collected by both CDC-CO_2 and Mosquito Magnet (Xue et al. 2008). Therefore, CDC-CO_2 should be compared with Mosquito Magnet in the southeastern Atlantic forest to evaluate the best available method of surveillance of adult females of mosquito species involved in the dynamics of parasite transmission to humans and sylvatic animals.

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Model selection of sensibility among collection sites

Models	Site 1 AIC (n)	Site 2 AIC (n)	Site 3 AIC (n)
1b (neutral model)	61	83	51
2b (control effect)	19	19	19
3b (multiple effects)	15	15	15
4b (CO_2 effect)	22	16	20
5b (1-octen-3-ol effect)	47	47	33
6b (synergic effect)	54	59	38

AIC: Akaike Information Criteria; CO_2: carbon dioxide; 1-octen-3-ol: octenol.

Model selection of abundance among collection sites

Models	Site 1 AIC (n)	Site 2 AIC (n)	Site 3 AIC (n)
1b (neutral model)	233	232	216
2b (control effect)	16	17	19
3b (multiple effects)	8	9	11
4b (CO_2 effect)	21	22	17
5b (1-octen-3-ol effect)	95	80	76
6b (synergic effect)	88	88	76

AIC: Akaike Information Criteria; CO_2: carbon dioxide; 1-octen-3-ol: octenol.