

Comparison of automatic traps to capture mosquitoes (Diptera: Culicidae) in rural areas in the tropical Atlantic rainforest

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In several countries, surveillance of insect vectors is accomplished with automatic traps. This study addressed the performance of Mosquito Magnet® Independence (MMI) in comparison with those of CDC with CO₂ and lactic acid (CDC-A) and CDC light trap (CDC-LT). The collection sites were in a rural region located in a fragment of secondary tropical Atlantic rainforest, southeastern Brazil. Limatus durhami and Limatus flavisetosus were the dominant species in the MMI, whereas Ochlerotatus scapularis was most abundant in CDC-A. Culex ribeirensis and Culex sacchettiae were dominant species in the CDC-LT. Comparisons among traps were based on diversity indices. Results from the diversity analyses showed that the MMI captured a higher abundance of mosquitoes and that the species richness estimated with it was higher than with CDC-LT. Contrasting, difference between MMI and CDC-A was not statistically significant. Consequently, the latter trap seems to be both an alternative for the MMI and complementary to it for ecological studies and entomological surveillance.

Key words: CDC-LT - Mosquito Magnet® - carbon dioxide - lactic acid - surveillance

Kairomones chemicals can be employed in mosquito automatic traps to improve their attractiveness (Service 1993). Carbon dioxide is one of the kairomones most largely used in Culicidae studies. Moreover, it can be used either alone or in combination with octenol, lactic acid, incandescent light or light-emitting diode (Hoel et al. 2007, Brown et al. 2008, Xue et al. 2008, Jawara et al. 2011).

Automatic traps are largely employed for monitoring mosquito vector-borne disease populations (Cohnstaedt et al. 2008, Bisevac et al. 2009, Calzolari et al. 2010), ecological studies (Forattini et al. 1991, Jones et al. 2004, Cardoso et al. 2011), biodiversity studies (Montes 2005) and species distribution (Hutchings et al. 2011). However, automatic traps perform differently depending on the model of the trap and the kairomones added to it (Gama et al. 2007, Cohnstaedt et al. 2008, Bisevac et al. 2009, Dusfour et al. 2010). Consequently, it is important to be aware of limitations when selecting a trap and the kairomones to add to it in order to get the best sampling performance for the study in focus (Gullan & Cranston 2005, Gama et al. 2007). Furthermore, surveillance personal need to be sure that the selected trap is capable of sampling the target species, allowing them to estimate abundance, composition, distribution and seasonality of mosquito populations present in an environment under certain ecological conditions.

The CDC light trap (CDC-LT) (Sudia & Chamberlain 1962) is the standard trap for surveillance and ecological studies on mosquito (Gomes et al. 1985, 1987, Hoel et al. 2009). Consequently, it has been largely employed in studies that aimed to compare the performance of different traps (Brown et al. 2008, Xue et al. 2008, Dusfour et al. 2010, Hiwat et al. 2011b), including in forested areas within the domain of Atlantic Forest biome (Laporta & Sallum 2011).

Currently, Mosquito Magnet® (MM) (Woodstream Corporation, Lititz, PA, USA) automatic traps are becoming an increasingly important instrument for mosquito surveillance (Bell et al. 2005). Studies using distinct models of MM were conducted in several countries to address the most efficient trap for sampling mosquito vector species in urban and rural areas (Brown et al. 2008, Dusfour et al. 2010, Hiwat et al. 2011a, Rubio-Palis et al. 2012). There is no record regarding the performance of MM in areas of the Atlantic rainforest. The major objectives of the present study were: (i) to assess the performance of the Mosquito Magnet® Independence (MMI) and (ii) to compare the capacity of the MMI to that of CDC associated with CO₂ and Lurex3® (lactic acid). A CDC-LT with incandescent light was employed as control. Comparisons among the traps were mainly based on the assessment of mosquito fauna present in areas situated within the Atlantic Forest domain. Field collections were carried out in three rural localities with different environmental characteristics at Agronomy Center for the Vale do Ribeira, Agronomic Institute of Campinas, Pariqueira Açú, state of São Paulo (SP), Brazil.

MATERIALS AND METHODS

Mosquitoes were sampled in rural areas interspersed with fragments of secondary forest at Agronomy Center for the Vale do Ribeira (Fig. 1). Three localities equidistant by 1 km were selected to install one MMI, one CDC with CO₂ and Lurex3® [CDC with CO₂ and lactic

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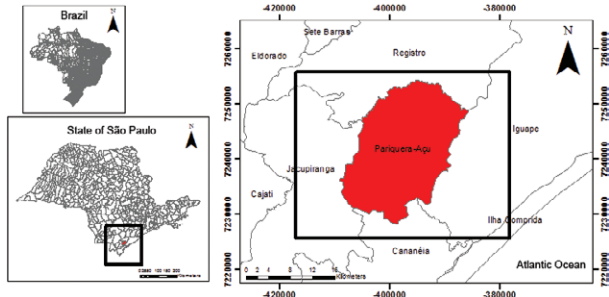


Fig. 1: location of the municipality of Pariqueira Açu, Vale do Ribeira, state of São Paulo, Brazil.

acid (CDC-A)] and one CDC-LT. Mosquito collections were carried out monthly, from 15:00 pm until 21:00 pm, from December 2010–November 2011. A 3 x 3 Latin square design was used to control environmental variations relative to the localities where traps were installed. Accordingly, each trap was placed in a specific locality in day 1 and in the following two days they were rotated in a way that each trap sampled all three localities in three consecutive days. The sampling effort of each trap per month was 18-h (6 h/per day in 3 consecutive days). Specimens were identified employing morphological keys proposed by Lane (1953) for the genus *Limatus*, Galindo et al. (1954) for the genus *Uranotaenia*, Correa and Ramalho (1956) for the subgenus *Phoniomyia* of *Wyeomyia*, Consoli and Lourenço-de-Oliveira (1994) for the *Aedini*, Forattini (2002) for the genus *Anopheles* and for the spissipes section of *Culex* (*Melanoconion*).

The CDC-LT possessed a motor powered by a 6-V battery, a head connected to a collecting metal chamber and a 3-W incandescent bulb. The CDC-A was operated by a similar system, with no incandescent bulb, but with CO₂ and lactic acid as attractive. A cartridge of the Lurex3® (American Biophysics Corporation) provided the lactic acid. Each cartridge contained 4.88 g of lactic acid incorporated to 13.8 g of a gel matrix. This mixture was stored inside a plastic tube, allowing the release of approximately 230 mg/day of lactic acid (Hoel et al. 2007). The CO₂ was provided by a metal cylinder with four and a half pounds (White Martins®), with an adjustable valve (Swagelok®) that released 450–500 mL of CO₂ per minute. The MMI used a mixture of propane and butane, which through its combustion releases CO₂, heat and water vapour (Hoel et al. 2009). All traps were installed at 1.5 m heights.

The statistical analyses were performed in the program R 2.12, using the packages Mvabund, Venneuler and Biodiversity R and the software SPSS v.13.0. Levene, Shapiro-Wilk and Kolmogorov-Smirnov tests were performed to explore the normality of abundance data. Indices of diversity were employed to address and compare the performance of the traps. The Rényi diversity index (Henderson 2006) that generalizes total richness ($\alpha = 0$), diversity (Shannon-Weiner $\alpha = 1$; Simpson-Yule $\alpha = 2$) and dominance (Berger-Parker $\alpha = \infty$) indices was employed to explore differences in species diversity among

traps. The Kruskal-Wallis (KW) test ($p > 0.05$) was used to assess differences among the Rényi diversity indices estimated by each trap and the Bonferroni test was utilised to perform multiple comparisons among the traps. Additionally, the similarity of species captured by the traps was estimated using the Jaccard and Sorensen index. The Veen diagram was constructed to illustrate the similarities and differences among the traps, because it shows both exclusive and shared species obtained by the traps. To assess potential associations among mosquito species and traps we employed the Pearson chi-square test (χ^2). The nonparametric test of second order Jackknife was used to estimate the expected species richness for each trap. Multivariate analyses of abundance values were employed to address both the effect of the traps and effect of the localities in the frequency of individuals of a species sampled by each trap. For the multivariate analyses, we employed a generalised linear model (GLM) implemented in the mvabund package of statistics program R 2.12 (Wang et al. 2012). The mvabund package uses a hypothesis test based on resampling to address potential factors associated with variables (Bálint et al. 2013). A GLM was employed by Warnick et al. (1990) to replace a model based on the distance of abundance. According to Wang et al. (2012), models based on distance do not clarify the effect of sampling units.

RESULTS

Six thousand three hundred and ninety one mosquitoes were captured with a 216-h sampling effort. Three hundred thirty six specimens could not be identified because they were damaged. The remaining 6,055 specimens were identified in 70 species or taxonomic units of 12 genera (Table I).

Results of the normality tests showed that the abundance data has a non-normal distribution. Accordingly, Levene's test obtained value $p = 0.001$, whereas in both Kolmogorov-Smirnov and Shapiro-Wilk tests was $p = 0.000$. These values indicated that the abundance data had a non-normal distribution.

The Pearson χ -square statistics showed potential association between CDC-LT and the tribe Culicini. Species from this tribe represented 78.9% of total mosquitoes captured by the trap ($\chi^2 = 2760.04$; $p < 0.00$). For the CDC-A, a positive association was found for representatives of the tribe Aedini with 58.3% of the total sampled by it, whereas a positive association was observed between Sabethini mosquitoes and MMI. This trap captured 36.1% of specimens.

The Rényi diversity index (Fig. 2) showed that the MMI found the highest values for the diversity indexes in comparison to CDC-LT and CDC-A, whereas results of the KW test indicated that the differences in the diversity indexes were statistically significant, i.e., richness ($KW\chi^2 = 14.88$, $p = 0.0006$), Shannon-Weiner diversity ($KW\chi^2 = 19.62$, $p = 0.00005$), Simpson-Yule diversity ($KW\chi^2 = 19.35$, $p = 0.00006$) and Berger-Parker dominance ($KW\chi^2 = 4.19$, $p = 0.00007$).

Results of the Bonferroni test indicated that differences in the diversity indexes were significant and mainly caused by the values estimated for the MMI in

TABLE I

Species and taxonomic units abundance per sampling trap in rural areas in the tropical Atlantic rainforest, southeastern Brazil

Species and taxonomic unit	CDC-LT (n)	CDC-A (n)	MMI (n)	Total (n)
Anophelinae				
<i>Anopheles costai</i> Fonseca & Silva Ramos	0	8	19	27
<i>Anopheles bellator</i> Dyar & Knab	0	1	0	1
<i>Anopheles cruzii</i> Dyar & Knab	0	1	1	2
<i>Anopheles galvaoi</i> Causey, Deane & Deane	1	0	0	1
<i>Anopheles triannulatus</i> (Neiva & Pinto)	1	0	0	1
Aedeomyiini				
<i>Aedeomyia squamipennis</i> (Lynch Arribalzaga)	2	1	0	3
Aedini				
<i>Ochlerotatus fulvus</i> (Wiedemann)	0	1	9	10
<i>Ochlerotatus hastatus/oligopistus</i> Dyar	0	5	1	6
<i>Ochlerotatus scapularis</i> (Rondani)	12	501	275	788
<i>Ochlerotatus serratus</i> (Theobaldi)	28	283	113	424
<i>Ochlerotatus serratus/nubilus</i>	7	133	34	174
<i>Ochlerotatus argyrothorax</i> Bonne-Wepster & Bonne	0	1	9	10
<i>Stegomyia albopicta</i> (Skuse)	0	0	1	1
<i>Psorophora cingulata</i> (Fabricius)	1	1	2	4
<i>Psorophora albigenu</i> (Peryassú)	-	78	186	264
<i>Psorophora albipes</i> (Theobald)	2	71	21	94
<i>Psorophora ferox</i> (Von Humboldt)	2	42	34	78
Culicini				
<i>Culex amazonensis</i> (Lutz)	9	2	11	22
<i>Culex chidesteri</i> Dyar	9	3	0	12
<i>Culex eduardoi</i> Casal & Garcia	2	0	0	2
<i>Culex nigripalpus</i> Theobald	94	36	70	200
<i>Culex quinquefasciatus</i> Say	0	0	2	2
<i>Culex</i> spp	66	34	5	105
<i>Culex</i> spp Coronator Complex	6	3	1	10
<i>Culex akritos</i> Forattini & Sallum	3	0	0	3
<i>Culex bastagarius</i> Dyar & Knab	7	0	0	7
<i>Culex faurani</i> Duret	2	1	1	4
<i>Culex ocosa</i> Dyar & Knab	1	0	0	1
<i>Culex pedroi</i> Sirivanakarn & Belkin	3	1	0	4
<i>Culex ribeirensis</i> Forattini & Sallum	432	86	285	803
<i>Culex sacchettiae</i> Sirivanakarn & Jakob	100	77	216	393
<i>Culex</i> spp Atratus Group	4	3	0	7
<i>Culex</i> spp Pilosus Group	3	1	3	7
<i>Culex</i> spp Melanoconion Section	26	3	0	29
<i>Culex spissipes</i> (Theobald)	1	0	0	1
<i>Culex vaxus</i> Dyar	10	0	0	10
<i>Culex zeteki</i> Dyar	4	0	0	4
Mansoniini				
<i>Coquillettidia albicosta</i> (Peryassú)	1	0	1	2
<i>Coquillettidia chrysonotum</i> (Peryassú)	43	167	518	728
<i>Coquillettidia hermanoi</i> (Lane & Coutinho)	0	0	1	1
<i>Coquillettidia venezuelensis</i> (Theobald)	28	12	50	90
<i>Mansonia humeralis</i> Dyar & Knab	0	1	0	1
<i>Mansonia indubitans</i> Dyar & Shannon	0	17	65	82
<i>Mansonia titillans</i> (Walker)	20	23	75	118
<i>Mansonia wilsoni</i> (Barreto & Coutinho)	0	0	3	3

Species and taxonomic unit	CDC-LT (n)	CDC-A (n)	MMI (n)	Total (n)
Sabethini				
<i>Limatus durhami</i> Theobald	14	259	545	818
<i>Limatus flavisetosus</i> de Oliveira Castro	1	21	323	345
<i>Runchomyia reversa</i> Lane & Cerqueira	0	6	10	16
<i>Runchomyia theobaldi</i> (Lane & Cerqueira)	0	2	0	2
<i>Sabethes ignotus</i> Harbach	0	0	1	1
<i>Wyeomyia felicia/pampeithes</i> (Dyar & Nunez Tovar)	0	3	34	37
<i>Wyeomyia bonnei</i> (Lane & Cerqueira)	0	1	0	1
<i>Wyeomyia davisi</i> (Lane & Cerqueira)	0	1	8	9
<i>Wyeomyia flabellata</i> (Lane & Cerqueira)	0	0	1	1
<i>Wyeomyia incaudata</i> Root	0	0	5	5
<i>Wyeomyia pallidoventer</i> (Theobald)	0	0	4	4
<i>Wyeomyia pilicauda</i> Root	0	1	3	4
<i>Wyeomyia confusa</i> (Lutz)	2	22	169	193
<i>Wyeomyia aporonoma</i> Dyar & Knab	0	0	30	30
<i>Wyeomyia coenonotus/tarsata</i>	0	0	1	1
<i>Wyeomyia howardi/luteoventralis</i>	0	0	2	2
<i>Wyeomyia melanocephala</i> Dyar & Knab	0	1	1	2
<i>Wyeomyia personata</i> Lutz	1	0	0	1
<i>Wyeomyia roucouyana/chalcocephala</i>	0	0	1	1
Uranotaeniini				
<i>Uranotaenia apicalis</i> Theobald	5	0	0	5
<i>Uranotaenia calosomata</i> Dyar & Knab	7	0	0	7
<i>Uranotaenia geometrica</i> Theobald	2	0	0	2
<i>Uranotaenia lowii</i> Theobald	2	0	0	2
<i>Uranotaenia mathesoni</i> Lane	25	0	1	26
<i>Uranotaenia pulcherrima</i> Lynch Arribálzaga	1	0	0	1
Total abundance	990	1,914	3,151	6,055
Total species/taxonomic units	42	41	46	70

CDC-A: CDC with CO₂ and lactic acid; CDC-LT: CDC light trap; MMI: Mosquito Magnet® Independence.

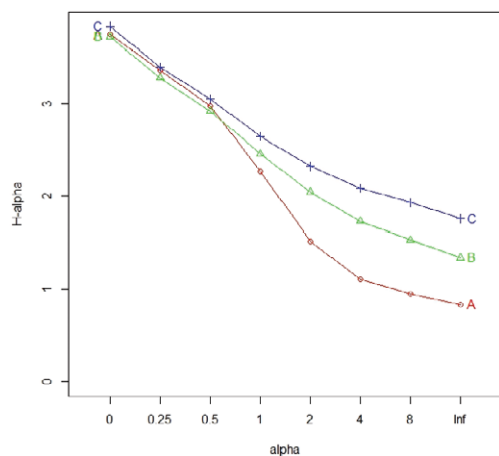


Fig. 2: Rényi index illustrating differences in diversities estimated by the traps. A: CDC light trap; B: CDC with CO₂ and lactic acid; C: Mosquito Magnet® Independence.

comparison to those for CDC-LT, whereas differences between CDC-A and MMI were not significant. Complete results of the KW and Bonferroni tests are in Supplementary data 1.

The extrapolated species richness using second-order Jackknife estimator to CDC-LT, CDC-A and MMI was 59, 67 and 77 species, respectively. Of the 42 species collected in CDC-LT, seven were singletons and eight were doubletons. In the CDC-A, 14 species were singletons and two were doubletons among 41 species collected, whereas the MMI captured 46 species with 13 singletons and three doubletons.

The values estimated with Jaccard ($c_j = 0.58$) and Sørensen ($C_n = 0.52$) indexes showed that species composition in the CDC-A was similar to that in the MMI. The Venn diagram (Fig. 3) illustrated species distribution in each trap and those species that were shared among them. A complete list of species captured in each trap and those shared by two or all traps are in Supplementary data 2.

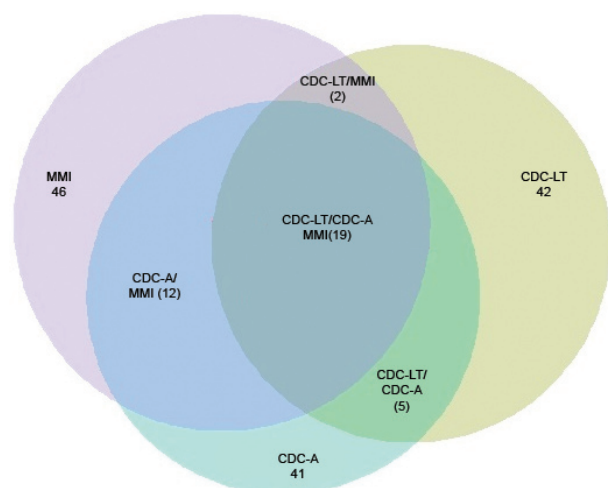


Fig. 3: Venn diagram illustrating the similarity of the mosquito species captured and shared between traps, CDC light trap (CDC-LT), CDC with CO₂ and lactic acid (CDC-A) and Mosquito Magnet® Independence (MMI), Pariquera Açu, Vale do Ribeira, state of São Paulo, Brazil.

Results of multivariate analysis of the abundance using a GLM showed that differences observed among the traps were statistically significant [Deviance of model's prediction (Dev) = 476.4, $p = 0.001$]. Comparing the values of abundance relative to the localities where the traps were installed, the variance values showed that differences were not significant (Dev = 19.0, $p = 0.76$) and that there was no interaction between the traps and the localities (Dev = 0.0, $p = 0.709$). Species that were more abundant in each trap are in Table II.

DISCUSSION

Results of the analyses carried out in the present study indicated that the MMI is the most efficient trap to capture mosquito species present in rural areas located in the deforested areas within the domain of tropical rainforests. Similar results were obtained by Brown et al. (2008), Hoel et al. (2009) and Dufour et al. (2010) with distinct models of MM automatic traps. These studies demonstrate that any model of MM trap is capable of removing large numbers of mosquito specimens from the environment, especially individuals from species with a great potential to become pest in environments impacted by human activities. This is the case of some species such as *Ochlerotatus scapularis*, *Culex ribeirensis* and *Coquillettidia chrysonotum*. These mosquitoes were abundant and had potential to become pests in both rural and urban environments (Forattini et al. 1991, 1993a, Cardoso et al. 2011).

Several models of MM were developed to effectively removing synanthropic mosquitoes from urban and rural environments (Cilek & Hallmon 2005). However, MM traps were also employed for surveillance of mosquito species with potential to transmit the West Nile virus in the United Kingdom (Hutchinson et al. 2007). In Denmark, the same trap was utilised to sample Cera-topogonidae insects in agricultural areas and thus to

monitor the circulation of the Schmallenberg virus. Infections by the Schmallenberg virus can cause congenital malformations and stillbirths in cattle and goats (Rasmussen et al. 2012). In Venezuela, studies employing MM traps showed that it was efficient for collecting *Anopheles nuneztovari* mosquitoes in a malaria-endemic area in the Amazon Basin (Rubio-Palis et al. 2012).

Results of the statistical tests indicated a significant difference regarding to diversity indexes obtained with both CDC-A and MMI in comparison to those with CDC-LT. Moreover, differences between CDC-A and MMI were not statistically significant. This can be explained by similarity between CDC-A and MMI, which was likely caused by the use of kairomones that promoted the capture of the same species. Forattini et al. (1987, 1993b) used carbon dioxide in CDC-LT to study the population dynamics, feeding behaviour and ecological characteristics of mosquito communities in a gradient of environments in the Vale do Ribeira. Accordingly, those authors showed that *Oc. scapularis* was abundant in rice plantation areas (Forattini et al. 1993b) and in anthropic environments. Additionally, the species showed capacity to invade, establish and disperse in domiciliary environments (Forattini et al. 1987).

As a result of the study, CDC-A and CDC-LT were similar regarding to species richness, but distinct in addressing both species composition and abundance. However, the CDC-A sampled a higher number of *Oc. scapularis* and *Ochlerotatus serratus*. These species can feed on mammals, including humans and other vertebrates and thus seems to be capable of participating in the transmission cycle of pathogens from rural to urban environments (Forattini et al. 1989, Laporta & Sallum 2011). It is noteworthy that only CDC-LT captured specimens of the genus *Uranotaenia*, corroborating the results of Xue et al. (2008). Species of the genus *Uranotaenia* are not anthropophilic and therefore were not attracted to kairomones employees in CDC-A and MMI. These chemicals were developed to attract insects that feed on humans and domestic animals and therefore they are not suitable to sample zoophilic species, for instance, *Uranotaenia* species that mostly feed upon amphibian blood (Cupp et al. 2004) that can be reservoirs of the eastern equine encephalitis virus in North America (Graham et al. 2012).

Laporta and Sallum (2011) investigated the potential of using carbon dioxide and octenol combined and separated as attractive in CDC-LT in a preserved Atlantic Forest area, in Cananéia, SP. As a result, authors showed that the addition of CO₂ in the CDC-LT increased both abundance and richness of mosquitoes sampled by CDC-LT. Contrasting, either octenol only or a combination octenol and CO₂ did not improve the results of CDC-LT. It is possible that the low concentration of octenol employed in the traps did not contribute to a synergistic effect with CO₂. Similar studies carried out in Florida, United States of America, to investigate the attractiveness of chemicals on mosquitoes showed that the performance of CDC-LT increased with the addition of CO₂ (Kline et al. 1990). Moreover, the lactic acid added to the

TABLE II
Results of multivariate analyses using a generalised linear model
to compare the values of abundance of mosquito species and taxonomic units obtained with each automatic trap

Taxonomic unit	Deviance	Pr (> Deviance)	Trap
<i>Ochlerotatus scapularis</i>	11.59	0.055	CDC-A
<i>Coquillettidia chrysonotum</i>	16.09	0.008	MMI
<i>Culex (Cux.)</i> spp	12.02	0.051	CDC-LT
<i>Culex (Mel.)</i> spp Melanoconion Section	17.17	0.005	CDC-LT
<i>Limatus durhami</i>	42.71	0.001	MMI
<i>Limatus flavisetosus</i>	41.20	0.001	MMI
<i>Mansonia indubitans</i>	12.82	0.036	MMI
<i>Phlegethontius albigenu</i>	13.57	0.027	MMI
<i>Uranotaenia calosomata</i>	13.99	0.019	CDC-LT
<i>Wyeomyia felicia/pampithes</i>	25.94	0.001	MMI
<i>Wyeomyia confusa</i>	38.97	0.001	MMI
<i>Wyeomyia aporonomia</i>	36.05	0.001	MMI

CDC-A: CDC with CO₂ and lactic acid; CDC-LT: CDC light trap; MMI: Mosquito Magnet® Independence; Pr: probability.

CDC-LT increased the potential of the trap to capture *Culex nigripalpus*, an important vector-borne species in North America.

Thus, MMI and CDC-A seems to be important traps for surveillance to the mosquito species. It is noteworthy that for the control of sucking species or synanthropic vector with potential role of pathogens to humans, the use of MMI, with carbon dioxide and lactic acid, is the most suitable. However, to realisation of collections in places with difficult access or stratification studies, for example, their use may be impaired. Accordingly CDC-A, which uses the same attractive may be an option to replace the MMI, as it can be more easily handled and compared to MMI relative for the ability to collection of the number and composition of the assembly of mosquitoes present in the focus of research.

These studies encouraging for further studies employed MM trap for surveillance of mosquito species involved in the parasite transmission to humans and ecological studies.

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