FIELD ASSESSMENT OF YEAST- AND OXALIC ACID–GENERATED CARBON DIOXIDE FOR MOSQUITO SURVEILLANCE

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ABSTRACT. Carbon dioxide (CO$_2$) sources improve the efficacy of mosquito traps. However, traditional CO$_2$ sources (dry ice or compressed gas) may be difficult to acquire for vector surveillance during military contingency operations. For this reason, a new and convenient source of CO$_2$ is required. Two novel CO$_2$ generators were evaluated in order to address this capability gap: 1) an electrolyzer that converts solid oxalic acid into CO$_2$ gas, and 2) CO$_2$ produced by yeast as it metabolizes sugar. The flow rate and CO$_2$ concentration produced by each generator were measured, and each generator’s ability to attract mosquitoes to BG-Sentinel™ traps during day surveillance and to Centers for Disease Control and Prevention light traps with incandescent bulbs during night surveillance was compared to dry ice and compressed gas in Jacksonville, FL. The electrolyzed oxalic acid only slightly increased the number of mosquitoes captured compared to unbaited traps. Based on the modest increase in mosquito collection for traps paired with the oxalic acid, it is not a suitable stand-in for either of the 2 traditional CO$_2$ sources. Conversely, the yeast-generated CO$_2$ resulted in collections with mosquito abundance and species richness more closely resembling those of the traditional CO$_2$ sources, despite achieving a lower CO$_2$ flow rate. Therefore, if dry ice or compressed gas cannot be acquired for vector surveillance, yeast-generated CO$_2$ can significantly improve trap capability.

KEY WORDS Vector surveillance, BG-Sentinel™, Centers for Disease Control and Prevention light trap, sugar-fermenting yeast, electrolyzed oxalic acid

INTRODUCTION

Successful vector-borne disease control requires accurate surveillance data. Without knowledge of an area’s arthropod vectors and their densities, control efforts may not be successful. Traps are integral to mosquito surveillance programs, as they directly provide the data to estimate mosquito density and diversity, population age-structure, and pathogen infection rates (Garrett-Jones 1964, Reisen and Pfuntner 1987, Gu et al. 2003, Kilpatrick et al. 2005, Eisen and Eisen 2008). Population data from trap surveillance provide key information for the development of disease risk assessment models (Diuk-Wasser et al. 2006), and the implementation of mosquito control measures (Amoo et al. 2008). While most traps deployed for mosquito surveillance are capable of attracting mosquitoes without an olfactant, the addition of a CO$_2$ source greatly improves the trap’s surveillance capability (Carestia and Savage 1967, Vythilingam et al. 1992). Dry ice and compressed CO$_2$ gas from canisters are commonly employed as baits for mosquito surveillance, with each source significantly augmenting the efficacy of the traps they are paired with (Newhouse et al. 1966, Carestia and Horner 1968). For military purposes, CO$_2$ canisters and dry ice for vector surveillance can be difficult to acquire during contingency operations in remote regions (AFPMB 2002, 2013), especially if continuous supplies are needed to sustain a long-term surveillance program. For this reason, alternative and easily acquired sources of CO$_2$ are required during contingency operations. Due to the nature of military operations, alternative CO$_2$ generators should be lightweight, durable, portable, easy to use, repair, and maintain, and be deployable for long periods. Most of these requirements would also make alternative CO$_2$ sources useful for public health programs throughout the developing world, where dry ice and compressed gas are unobtainable or too expensive (Oli et al. 2005, Moncaz et al. 2013).

The risk of vector-borne disease transmission in an environment is dependent on both the species in an area and their population density (Olson et al. 1979, Eldridge 2004, Scott and Morrison 2004). Consequently, for a novel source of CO$_2$ to be a useful stand-in for surveillance when dry ice and compressed CO$_2$ are unavailable, it must attract the same species of mosquitoes as traditional CO$_2$ sources, at comparable densities, and also attract species that unbaited traps may fail to collect. In this study, the ability of 2 novel sources of CO$_2$ to meet these requirements was assessed during day and night...
Carbon dioxide (CO2) sources improve the efficacy of mosquito traps. However, traditional CO2 sources (dry ice or compressed gas) may be difficult to acquire for vector surveillance during military contingency operations. For this reason, a new and convenient source of CO2 is required. Two novel CO2 generators were evaluated in order to address this capability gap: 1) an electrolyzer that converts solid oxalic acid into CO2 gas, and 2) CO2 produced by yeast as it metabolizes sugar. The flow rate and CO2 concentration produced by each generator were measured, and each generator's ability to attract mosquitoes to BG-Sentinel™ traps during day surveillance and to Centers for Disease Control and Prevention light traps with incandescent bulbs during night surveillance was compared to dry ice and compressed gas in Jacksonville, FL. The electrolyzed oxalic acid only slightly increased the number of mosquitoes captured compared to unbaited traps. Based on the modest increase in mosquito collection for traps paired with the oxalic acid, it is not a suitable stand-in for either of the 2 traditional CO2 sources. Conversely, the yeast-generated CO2 resulted in collections with mosquito abundance and species richness more closely resembling those of the traditional CO2 sources, despite achieving a lower CO2 flow rate. Therefore, if dry ice or compressed gas cannot be acquired for vector surveillance, yeast-generated CO2 can significantly improve trap capability.
surveillance. One novel source of CO₂ was generated by a fermentation chamber, in which yeast metabolized sucrose. This source had been shown to attract various mosquito species in field and laboratory conditions (Saitoh et al. 2004, Oli et al. 2005, Smallengange et al. 2010, Obenauer et al. 2013, Steiger et al. 2014), but had not been implemented in the surveillance of day-feeding mosquitoes or compared to other novel sources of CO₂. The other novel source involves a chemical reaction between water, oxalic acid, and an electrical current to generate CO₂. To our knowledge, this technology has not yet been field tested or compared to other sources of CO₂.

MATERIALS AND METHODS

CO₂ sources

Four CO₂ sources were evaluated and compared in this study. Dry ice and pressurized CO₂ gas from a cylindrical tank are conventional sources of CO₂ gas, and were compared to the 2 novel CO₂ generators. For the appropriate treatments, approximately 2 kg of dry ice was placed in a thermos to provide CO₂ gas for the duration of the 6-h and 12-h trapping periods. The dry ice was expected to release 0.55 m³ CO₂/kg (Caldwell et al. 2006), and sublime in 24 h at an average flow rate of 763.88 ml/min. This estimation provides a reference point for the expected amount of CO₂ produced by dry ice, which would also be influenced by the environmental conditions in the surveillance area, and is only used to demonstrate that dry ice is releasing CO₂ gas at higher flow rates than all other sources being evaluated. Carbon dioxide gas was also supplied from 1 20-lb CO₂ tank (Praxair Inc., Danburg, CT). A gauge was placed on the valve of the tubing under water and observing for gas bubbles. Day sampling was conducted using BG-Sentinel™ (BGS) (BioGents, Regensburg, Germany), which is designed to attract Aedes aegypti.

This airtight seal of each generator was tested before each trapping period. The CO₂ generated by electro-stripping oxalic acid was produced by a Moustiq-Air™ CO₂ generator (Med-e-Cell, San Diego, CA) powered with a lithium iron (Li-Fe) battery (Tenergy Corporation, Fremont, CA). The Moustiq-Air is an electrochemical generator that electro-strips CO₂ from oxalic acid. To operate, approximately 500 ml of water are added to the reservoir holding the oxalic acid feed-ring, and a direct current power source is connected to the 2 terminals that power the electrolyzer. A 0.5-cm inner diam polyethylene tube is inserted through the gas exhaust port in the lid of the unit, allowing CO₂ delivery (Fig. 1B). The Moustiq-Air was claimed by the manufacturer to generate 12 liters of CO₂ per hour, at a flow rate of 200 ml/min. The functionality of Moustiq-Air was tested before and after each trap period by submerging the open end of the tubing under water and observing for gas bubbles.

Flow rates

Under laboratory conditions (25.9 ± 0.01°C, RH 52.1 ± 2.0%), the flow rates of the oxalic acid and yeast CO₂ sources were measured using an inverted beaker filled with water and held at the waterline in a tub of water, just above the output of the polyethylene tubing that delivered the gas from the generator. The gas that each generator produced displaced water from inside the beaker, so the amount of displaced water (ml) in 1 min was the observed flow rate (ml/min). The 1st measurement began 15 min after the yeast and sugar slurry was mixed, and subsequent recordings were made hourly over 6 h. The measurements were replicated twice each day, over the course of 4 days (n = 8). In addition to measuring flow rate, the concentration of CO₂, measured in parts per million (ppm), in the atmosphere was measured 30 cm from the polyethylene tube outputs of the Moustiq-Air and the yeast generator using an EasyView® 80 CO₂ Analyzer (Extech Instruments, Nashua, NH) and was compared to the atmospheric concentration of CO₂ 30 cm from a thermos containing dry ice every 1 min over a 6-h period.

Study areas

Day trapping occurred in a residential neighborhood of Jacksonville, FL. Approximately 70% of the selected property was bordered by a natural area composed primarily of native, naturalized, and ornamental vegetation prone to flooding that creates ephemeral pools of standing water. Day sampling was conducted using BG-Sentinel™ (BGS) (BioGents, Regensburg, Germany), which is designed to attract Aedes aegypti.
(Linnaeus) and Ae. albopictus (Skuse) (Williams et al. 2006, Kawada et al. 2007). The BGS has been observed to be more attractive to Ae. albopictus than other traps (Farajollahi et al. 2009). While the trap can also be used with an attractant that mimics human odors, we only evaluated the traps with the CO$_2$ attractants. The BGS traps were placed along the property’s perimeter, spaced 20 m apart to avoid the host cues from the different CO$_2$ sources from mixing (Gillies and Wilkes 1970). Day trapping involved 4 treatments paired with the BGS: 1) no CO$_2$ source, in which the trap was placed without being baited; 2) CO$_2$ from dry ice, where 2 kg of dry ice were placed in an insulated thermos with ventilation holes and hung 0.5 m above the BGS; 3) CO$_2$ from yeast-fermenting sugar, where 2 3-liter bottles containing the yeast/sugar mixture provided CO$_2$ through polyethylene tubing placed near the trap’s opening; and 4) CO$_2$ from electrolyzed oxalic acid, generated by the Moustiq-Air powered by a 40-Ah Li-Fe battery, delivered to the opening of the BGS with polyethylene tubing. A 4 x 4 Latin square design was implemented over 8 days, so that each of the 4 CO$_2$ sources was placed at each of the 4 locations for 2 days of trapping. The Moustiq-Air could only operate for 6 h when the 40-Ah battery was powering it; therefore, day surveillance was conducted from 0800–1400 h.

Night trapping was conducted at Naval Air Station (NAS) Jacksonville, FL, in an undeveloped area near an antenna placement. The area was part of a greater nature preserve at the air station, characterized by native vegetation, with a canopy composed primarily of slash pine (Pinus elliottii Engelmann) and an understory dominated by saw palmetto (Serenoa repens W. Bartram). The area is frequently flooded following rains, providing pools of standing water for larval mosquito habitat. The Centers for Disease Control and Prevention (CDC) light traps with
incandescent bulbs were implemented during the night surveillance since the traps are designed to sample night-feeding mosquitoes that are attracted to light. The light traps were hung approximately 1.5 m above the ground and spaced 20 m apart (Gillies and Wilkes 1970). The CDC light traps were deployed overnight for 12 h from 1830–0630 h. Five CO$_2$ sources were evaluated during the night sampling: 1) no CO$_2$ source, in which the CDC light trap was placed without being baited; 2) CO$_2$ from dry ice, where 2 kg of dry ice was placed in an insulated thermos with ventilation holes cut into the bottom and hung 0.25 m above the trap; 3) CO$_2$ gas from a 20-lb CO$_2$ canister (Praxair Inc.) delivered at approximately 250 ml/min by a polyethylene tube to approximately 5 cm from the trap entrance; 4) CO$_2$ from yeast-fermenting sugar, where 2 3-liter fermentation bottles delivered CO$_2$ to the trap via polyethylene tubing to the trap entrance (Fig. 1C); and 5) CO$_2$ from electrolyzed oxalic acid generated by the Moustiq-Air powered by an 80-Ah Li-Fe battery, delivered via polyethylene tubing to the trap entrance (Fig. 1D). A 5 × 5 Latin square design was implemented over 20 nights, so that each treatment was placed at each of the 5 trap locations 4 times. All mosquitoes collected during the sampling periods were identified to species with taxonomic keys. Nightly temperature and rainfall were recorded during the study. The surveillance data set for both day and night surveillance periods consisted of mosquito catches (abundance) and number of species present (species richness).

**Statistical methods**

All statistical tests were performed in Intel® Visual Fortran Composer XE 2013 (Intel Corporation, Santa Clara, CA) with α = 0.05. In a preliminary analysis with goodness-of-fit tests, the Kolmogorov–Smirnov test (Smirnov 1939) showed that the data sets were nonnormal, and the Bartlett test (Bartlett 1937a, 1937b) showed nonhomoscedastic behavior (nonhomogeneity of variances). Hence, nonparametric Kruskal–Wallis (K-W) hypothesis tests (Kruskal and Wallis 1952) were conducted in this study.

A 2-way K-W hypothesis test was utilized to assess differences in CO$_2$ flow rates (ml/min) between the yeast- and oxalic acid–generated CO$_2$ at the 7 observation times (hour 0–6). A nonparametric 1-way K-W test assessed the effect of the surveillance date on mosquito catch rate (number of individuals) and richness (number of species). A 2-way K-W test assessed differences in catch rate and species richness among treatments, among positions, and the treatment × position interaction. Additional 2-way K-W tests were also conducted to determine how each treatment, the trap placement, and the interaction of the treatment and location affected the number of *Anopheles* spp. and *Ae. albopictus* captured during the night and the day surveillance, respectively. These species were selected for individual analysis due to their ability to transmit malaria and dengue virus, respectively. Tukey multiple-comparisons tests were conducted to identify the specific variables that were significantly different from each other for each K-W test (Zar 1999).

**RESULTS**

A single yeast-generator bottle was found to generate gas at a greater rate (ml/min) than the electrolyzed oxalic acid (χ$^2$ = 37.38, df = 1, P < 0.0001). The flow rate produced by the electrolyzed oxalic acid was calculated to be on average 27.45 ± 1.72 ml/min, with the flow rate never exceeding 33 ml/min during the 7 h of operation, whereas a single yeast-fermentation bottle generated an average flow rate of 55.15 ± 9.08 ml/min, and a maximum average flow rate of 79.2 ml/min. Overall, the flow rate of both generators increased with time (χ$^2$ = 6.04, df = 6, P < 0.0001), and the yeast generator’s output increased at a faster rate than the oxalic acid (χ$^2$ = 2.63, df = 6, P = 0.02) (Fig. 2).

The baseline atmospheric CO$_2$ concentration recorded in the laboratory by the EasyView 80 CO$_2$ Analyzer was measured at 477.96 ± 1.65 ppm during a 6-h period. The average concentration of CO$_2$ measured 30 cm from the output of the electrolyzed oxalic acid during 6 h of operation was 874.27 ± 2.74 ppm. The average concentration of CO$_2$ measured 30 cm from the output of a single yeast fermentation tank over 6 h was 2,365.54 ± 46.38 ppm. The average concentration of CO$_2$ dry ice released into the atmosphere 30 cm from the ventilations holes of the insulated thermos could not be measured with the equipment we had available. The CO$_2$ analyzer
had a maximum detection limit of 6,000 ppm, which the dry ice exceeded during the 6-h observation. Therefore, the average CO₂ concentration of the atmosphere 30 cm from the dry ice was >6,000 ppm. The time-specific concentration of CO₂ generated by each source is presented in Fig. 3.

Results of the nonparametric K-W test demonstrated mosquito abundance and species richness were not affected by the date of collection during the day surveillance. However, the date that the night surveys were conducted significantly influenced the mosquito abundance ($\chi^2 = 36.11$, df = 19, $P = 0.01$), but not species richness. Tukey groupings showed a general increase in the mosquito abundance over time, until abundance peaked (August 28) and then declined (Fig. 4).

A total of 21 species of mosquito, in 9 genera, were collected between the day and night surveys (Table 1). Results of the nonparametric K-W tests showed that, for both the BGS and the CDC light trap, trap location did not influence mosquito abundance or species richness. Additionally, no significant treatments × position interactions were observed for the day or night trapping on mosquito abundance or species richness.

For the BGS trap, the treatment type influenced the number of mosquitoes trapped ($\chi^2 = 4.57$, df = 3, $P = 0.009$) and the number of species attracted ($\chi^2 = 5.72$, df = 3, $P = 0.003$). Tukey post hoc tests demonstrated that the addition of a CO₂ source to the BGS resulted in a greater number of mosquitoes collected compared with traps that lacked CO₂. Dry ice outperformed the 2 novel sources of CO₂, while the yeast outperformed the oxalic acid (Fig. 5A, 5C).

The treatment type also significantly influenced the number of mosquitoes trapped ($\chi^2 = 12.18$, df = 4, $P < 0.0001$) and the number of species collected ($\chi^2 = 9.98$, df = 4, $P < 0.0001$) by the CDC light traps. Tukey post hoc tests demonstrated that the addition of a CO₂ source significantly increases the number of mosquitoes a CDC light trap attracts, with dry ice outperforming all other sources, while the yeast outperformed the oxalic acid but not the CO₂ tank (Fig. 5B). Additionally, the CO₂ sources increased the number of species that are attracted to the light trap, with the dry ice attracting the greatest number of species, followed by the CO₂ tank. The CO₂ generated from yeast was found to attract more species of mosquitoes than the CO₂ from oxalic acid, and both yeast- and oxalic acid–generated CO₂ outperformed unbaited traps (Fig. 5D).

The location of the trap within each study area did not influence the number of Anopheles spp. collected during night surveillance or the number of Ae. albopictus collected during the day surveillance. Additionally, no significant interactions were observed between treatment type and location during the night and day surveillance of the Anopheles spp. or Ae. albopictus, respectively. There was an effect of the treatment on the number of Anopheles spp. collected ($\chi^2 = 10.9779$, df = 4, $P < 0.0001$), where each CO₂ source increased the number of Anopheles spp. sampled by baited traps compared to the unbaited control, with dry ice providing the greatest improvement, followed by the CO₂ tank, then yeast, and finally oxalic acid (Table 2). However, when species of Anopheles were independently evaluated the treatment was only found to affect the number of An. crucians (Wiedemann) ($\chi^2 = 8.32$, df = 4, $P < 0.0001$) attracted by each trap, while the addition of a CO₂ source had no effect on the number of An. quadrimaculatus (Say) collected (Table 2). Finally, the treatment type did not affect the number of Ae. albopictus collected by the BGS traps during the day surveillance (Table 2).

**DISCUSSION**

Since there was no effect of location on the traps’ surveillance efficacy, the mosquito populations at each site appeared homogeneously distributed throughout each environment. The lack of an effect of trap date at the day trapping location, in suburban Duval County, FL, suggests the mosquito populations did not fluctuate during the study, nor did the species...
composition in the area change in response to weather patterns or from our sampling efforts. In contrast, the mosquito populations from the natural area of NAS Jacksonville, FL, showed a strong dependence on time. This was likely an effect of the local environmental conditions, since the mosquito populations appeared to have increased following rains that flooded the area.

![Fig. 4. Bar plots showing average numbers of mosquitoes (± SD) captured each night by Centers for Disease Control and Prevention light traps from August 12, 2013, to September 16, 2013, at Naval Air Station Jacksonville, FL. Different letters above the date represent significant differences between the average number of mosquitoes collected in each trap, as determined through a Tukey post hoc analysis. The environmental conditions recorded at the field site during the study are also represented. Black triangles represent dates that rain was recorded (mm).](image)

Table 1. Total number of each mosquito species collected during day surveillance with BG-Sentinel™ traps, over 8 days (0800–1400 h) and during night surveillance with Centers for Disease Control and Prevention light traps, over 20 nights (1830–0630 h) when baited with CO₂ generated by dry ice, a pressurized CO₂ tank (Tank), a yeast and sugar solution (Yeast), and electro-stripped oxalic acid (OA).

<table>
<thead>
<tr>
<th>Species</th>
<th>Day Total</th>
<th>Night Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry ice</td>
<td>Yeast</td>
</tr>
<tr>
<td>Aedes albopictus</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>Ae. atlanticus</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Ae. sollicitans</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ae. triseriatus</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ae. fulvuspellus</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ae. infirmus</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>Anopheles crucians</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>An. quadramaculatus</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Coquillettidia perturbans</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Culex erreticus</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cx. nigripalpus</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Cx. quinquefasciatus</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cx. restuans</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Culiseta melanura</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mansonia dyari</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ms. titillans</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Psorophora ciliata</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ps. columbiae</td>
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<td>0</td>
</tr>
<tr>
<td>Ps. ferox</td>
<td>25</td>
<td>4</td>
</tr>
<tr>
<td>Uranotaenia lowii</td>
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<td>0</td>
</tr>
<tr>
<td>Ur. sapphirina</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>71</td>
<td>26</td>
</tr>
</tbody>
</table>
As expected, the addition of any CO$_2$, regardless of its source, improved the BGS and the CDC light trap’s ability to collect mosquitoes (Carestia and Savage 1967, Vythilingam et al. 1992). Nevertheless, dry ice outperformed all other CO$_2$ sources, resulting in a 13.5-fold increase in the average number of mosquitoes collected relative to unbaited BGS traps and a 5.9-fold increase relative to unbaited CDC light traps. Since the attractiveness of CO$_2$-baited traps is positively correlated with CO$_2$ flow rate (Carestia and Horner 1968), the high rate of CO$_2$ release during dry ice sublimation is likely the cause of the drastic improvement for both traps’ surveillance ability when baited with dry ice compared to the other treatments. The dry ice also yielded the greatest species richness, providing a more accurate picture of mosquito diversity in an environment compared with other CO$_2$ sources.

The CO$_2$ generated from electro-stripped oxalic acid increased the number of mosquitoes captured by 1.9-fold compared to the unbaited BGS during day surveillance and by 1.2-fold compared to unbaited CDC light traps during night surveillance. However, during day and night surveillance it resulted in very low catches compared to all other CO$_2$ sources. Additionally, the flow rate in which the oxalic acid produced CO$_2$ was about 10% of what the manufacturer claimed it should generate. The observed flow rate of the oxalic acid was also substantially less than that of the yeast-fermented CO$_2$. Therefore, it is likely the low numbers of mosquitoes attracted by the oxalic acid is due to the low flow rate achieved during this evaluation.

The CO$_2$ produced by the yeast-fermenting sugar outperformed the other novel CO$_2$ source, but did not compare favorably to the 2 traditional
sources. Despite the lower catch rate, the yeast-generated \( \text{CO}_2 \) is expected to provide a useful alternative when dry ice and \( \text{CO}_2 \) tanks are unavailable, since it improved the total number of mosquitoes captured by the BGS trap by 3.3-fold during day surveillance and the CDC light trap by 3.6-fold during night surveillance. It also attracted the relatively common species in the environment that were also collected by traps baited with dry ice. The mosquito species not collected by traps baited with yeast-generated \( \text{CO}_2 \) were rarely captured by the other \( \text{CO}_2 \) sources, so those not attracted by yeast are expected to occur in the environment at such low densities that they would pose a minimal threat to public health (Olson et al. 1979, Scott and Morrison 2004). The flow rate achieved by the yeast mixture in this study did not match what was observed by Smallengange et al. (2010) during field trials, even though the same ratio of yeast and sugar was evaluated here. The yeast used in this study may have influenced this, as could have the different environmental conditions under which each was tested. However, we produced similar flow rates to what Steiger et al. (2014) produced using a similar ratio of sugar and yeast, under similar laboratory conditions. Despite inconsistencies reported among these studies (Smallengange et al. 2010, Steiger et al. 2014), it is important to note that even at flow rates less than that expected from dry ice, the yeast generator still attracts a strong representation of the mosquito species in the environment at rates that are expected to provide adequate information to determine which vectors are present in an area. Moreover, the simplicity and speed that \( \text{CO}_2 \) can be generated with yeast, sugar, and water in a plastic bottle has advantages for fieldwork in remote localities. Therefore, in the absence of other \( \text{CO}_2 \) sources, yeast-generated \( \text{CO}_2 \) can be expected to provide a reliable alternative for surveillance.

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