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Short Communication

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A Comparative Study of Mosquito and Sand Fly (Diptera: Psychodidae: Phlebotominae) Sampling Using Dry Ice and Chemically Generated Carbon Dioxide From Three Different Prototype CO_2 Generators

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Abstract

A comparative study was conducted to test the efficiency of Centers for Disease Control and Prevention (CDC) light traps baited with either dry ice or carbon dioxide (CO_2) produced from one of three different sources in collecting mosquitoes (Diptera: Culicidae) in Thailand. Treatments consisted of dry ice pellets, CO_2 gas produced from one of three prototype CO_2 generator systems (TDA, CUBE, Moustiq-Air Med-e-Cell - MEC), and a CDC light trap without a CO_2 source. The best performing prototype from Thailand was then tested in collecting sand flies (Diptera: Psychodidae: Phlebotominae) in Greece. A total of 12,798 mosquitoes and 8,329 sand flies were sampled during the experimentation. The most prevalent mosquito species collected in Thailand were: *Culex vishnui* Theobald > *Anopheles minimus* Theobald > *Culex tritaeniorhynchus* Giles > *Anopheles sawadwongporni* Rattanarithikul & Green. By far the most prevalent sand fly species collected in Thessaloniki was *Phlebotomus perfiliewi* Parrot followed by *Phlebotomus tobbi* Adler and Theodor and *Phlebotomus simici* Nitzulescu. In general, the TDA treatment was the only treatment with no significant difference from the dry ice-treatment in mean trap catches. Although dry ice-baited traps caught higher numbers of mosquitoes and sand flies than the TDA-baited traps, there was no difference in the number of species collected. Results indicate that the traps baited with the TDA CO_2 generator were as attractive as traps supplied with dry ice and, therefore, the TDA CO_2 generator is a suitable alternative to dry ice as a source of carbon dioxide for use with adult mosquito and sand fly traps.

Key words: mosquito trapping, sand fly trapping, Phlebotomus, CO_2, dry ice, carbonate-acid CO_2 source

Carbon dioxide is one of the most important cues used by blood feeding arthropods for detecting host location. In mosquitoes (Diptera: Culicidae) and sand flies (Diptera: Psychodidae: Phlebotominae), CO_2 is involved in their activation and attraction from a long range in combination with other visual and odorous organo-chemical cues (Takken 1991, Takken and Knols 2010). Traps for capturing blood-seeking Dipterans are commonly baited with CO_2 to increase sampling efficiency and specificity. The Centers for Disease Control and Prevention (CDC) light traps baited with CO_2 in the form of dry ice or carbon dioxide cylinders are used extensively for both mosquito (Gillies 1980) and sand fly sampling (Alten et al. 2015) and are important tools for vector and disease surveillance programs globally. However, dry ice is difficult to obtain in many locations and CO_2 cylinders can be costly and difficult to transport due to extensive weight (Sukumaran et al. 2016) and special handling (special storage and disposal procedures). For these reasons, alternative routes of CO_2 production have been developed including the production through microbial metabolism (yeast-fermenting CO_2 production, Aldridge et al. 2016) as well as chemical production of CO_2, i.e., through the use of carbonates and or bi-carbonates and weak acids in aqueous solution (Burkett-Cadena et al. 2015). A recent publication showed high potential of carbonate-acid CO_2

sources to attract mosquitoes and black flies (Burkett-Cadena et al. 2015); however, this technique needs to be further studied and tested on a wider range of blood-feeding arthropods.

The objective of this research endeavor was to test the efficiency of CDC light traps baited with either dry ice or carbon dioxide (CO_2) produced from one of three prototype CO_2 generator systems (TDA, CUBE, Moustiq-Air Med-e-Cell - MEC) in collecting mosquitoes in Mae Sot District (Tak Province) and Muang District (Kamphaeng Phet Province), Thailand. The best performing CO_2 generator was then tested in Thessaloniki Regional Unit, Greece in collecting sand flies.

Materials and Methods

Study Sites

Thailand

Field experiments were conducted in two locations in Thailand. The first location was a rural area in Mae Sot District (Tak Province), endemic in malaria with approximately 160,000 people (Tainchum et al. 2014). Experiments were conducted in three villages (Khun-Huay, Tham-Suea, and Pa-Deh) in June 2014 targeting primarily *Anopheles* mosquitoes. Houses in these villages are not fully enclosed by walls and doors. Residents are both Thai and Karen tribe who migrated from Myanmar and are mainly farmers cultivating rice, corn, and soybean and rearing livestock. Average temperature and relative humidity during collection period in June was 26.7°C (range 25.1–27.2) and 80% RH (range 62–91). The second location was an urban environment in Muang District (approximately 213,000 people), Kamphaeng Phet Province (KPP). KPP is a dengue endemic area north of Bangkok (Endy et al. 2004, Bhoomiboonchoo et al. 2014) and all experiments were conducted in three villages in Nong-Pling sub-district in November 2014 targeting urban mosquito species including the common dengue vector, *Aedes aegypti*. Average temperature and relative humidity in KPP in November was 26.8°C (range 24.6–29.5) and 76% RH (range 67–91). Additional semifield experiments were conducted at KPP in large mosquito enclosures (LMEs) located at the Department of Entomology, Armed Forces Research Institute of Medical Sciences (AFRIMS 16o 30′ 27″ N, 99o 31′ 37″ E). Each LME (16.8 × 12 × 5.5 m) was covered with polyester netting thereby allowing the free entry of both wind and precipitation and creating climatic conditions within the enclosures that mimic ambient conditions. The roof was covered with thick white polyethylene plastic for protection from intense seasonal rains.

Greece

All studies were conducted in three medium, multianimal facilities with high levels of sand fly activity (Chaskopoulou et al. 2016) in a suburban area on the eastern outskirts of Thessaloniki. The Regional Unit of Thessaloniki occupies the center of Central Macedonia Region in Northern Greece and is the second largest city in the country with a population 1.5 million people. The city is endemic to canine leishmaniasis (CanL) with highest frequency of transmission around and along the outskirts of the city where most farming and agricultural activities are concentrated (Chaskopoulou et al. 2016). The mean annual precipitation and temperature is 478 mm and 16°C, respectively, with 67% relative humidity.

Carbon Dioxide Generator Prototypes

Three prototype generators were tested (Fig. 1) that used different mechanisms for CO_2 production. All generators were designed to produce a CO_2 release rate that would attract anthropophilic vector species (250–400 cc/min).

Prototype 1

The TDA Research, Inc., CO_2 generator uses an acid-base reaction for CO_2 production. The acid-base reaction is generated by mixing food-grade chemicals (sodium bicarbonate and malic acid) with water at a metered rate to produce average CO_2 rates of 400 cc/min over 12 h. These chemicals do not require special storage or shipping methods and are considered environmentally safe. Each generator shares the 6V battery with the light trap without a significant increase in power consumption. After a run, the system contains a neutralized solution with some solids floating on top. The remaining solution is safe and environmentally non-hazardous. However, any wastes, in the United States or elsewhere, should be disposed in accordance with federal, state, local, and installation regulations. The TDA device is provided in a case with all consumables and accessories necessary to operate for three surveillance periods (12 h each) except water, which can be acquired locally at any quality.

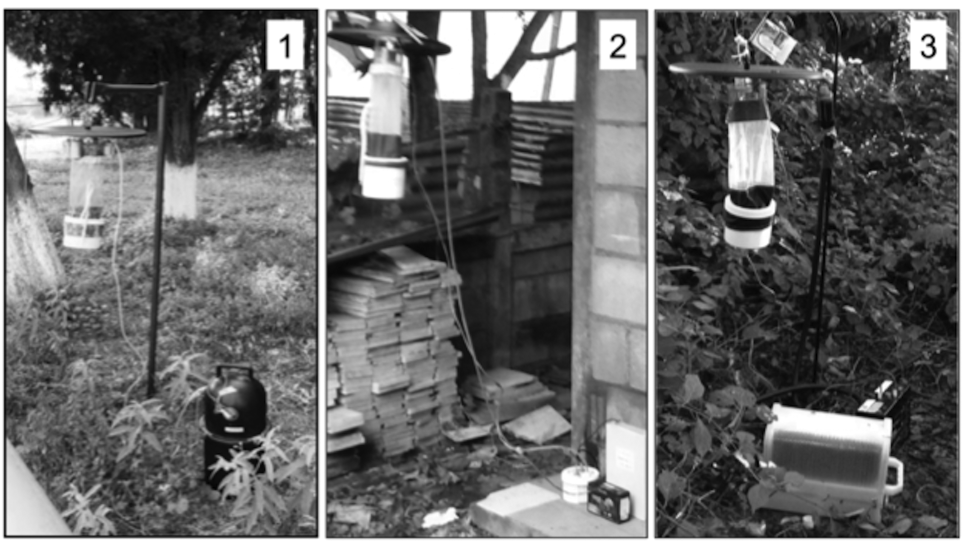

Fig. 1. CDC light traps with CO_2 configurations (1) TDA CO_2 generator, (2) with MEC CO_2 generator, and (3) with CUBE CO_2 generator.

Prototype 2

Moustiq-Air by Med-e-Cell (MEC) produces CO_2 by electro-stripping CO_2 from a carboxylated organic compound (oxalic acid). This electrochemical process is clean, controllable, and requires only water and a DC power source, such as a battery or wall plug-in. The starting material, oxalic acid, is unique to this vendor and is considered hazardous material. The MEC standard kit contains oxalic acid dihydrate feed ring(s), a generator in an individual container, 3.2 V LiFeP04 battery, charger, cables, outlet plug, and plastic tubing. The by-products of the reaction must be disposed of in a controlled manner. As provided, the MEC produces CO_2 rates of approximately 250 cc/min. Water must be acquired locally.

Prototype 3

The CUBE Technology, Inc. generator uses a combustion reaction to produce CO_2 using a compact Micro-Furnace that can operate on fuels such as diesel fuel or Jet Propellant 8 (JP-8). This combustion reaction produces CO_2 at an average rate of 400 cc/min. The generator also has an electrical outlet to drive the light trap. The system uses disposable filters, unique to this product. After each cycle, these can be disposed of in regular trash. The CUBE device is provided in a case with all accessories, except fuel, necessary to provide three surveillance periods, including a 12V rechargeable battery. Fuel must be acquired locally.

Experimental Design

Thailand

Field

The standard miniature CDC light trap (Model 512, John Hock, Gainesville, FL, USA) was used for capturing adult mosquitoes. Five different combinations/configurations of the trap were tested: CDC light trap alone, CDC light trap-TDA, CDC light trap-CUBE, CDC light trap-MEC, CDC light trap-dry ice in the form of dry ice pellets (3 kg of dry). The CDC traps were hung on either low trees or metallic posts (trap opening approximately 1.5 m from the ground) and the CO_2 sources were placed on the ground right below the trap and CO_2 output was delivered by tubing above the trap fan. The only exception to this was the dry ice holding container, which was hanged right above the trap. In Tak Province, trapping assessment was performed in each village using a Latin square design with the five trap combinations rotated through five locations over 5 d of trapping. Each day, sampling commenced at 1700 until 0900 on the next day (16 h). In KPP villages, the experiments were performed exactly as described above. The houses sampled were at least 20 m apart in order to minimize the overlapping of attractants generated from the various CO_2 sources. Specimens collected from both study sites were brought back to the field station, counted, sexed, and identified to species level (Rattanarithikul et al. 2006).

Semifield

CO_2 generators were tested in three LMEs against *Anopheles dirus* (AFRIMS strain), the major malaria vector in the region. Two types of CO_2 generators (TDA, MEC) were used in this study. The CUBE generators had repeated failures during the field studies and were excluded from the remaining collections. One CO_2 generator plus CDC light trap was directly compared to either dry ice plus the light trap (dry ice) or the light trap alone, placed randomly inside the LMEs for a total of three replicates for each comparison. For each trap period or replicate, a total of 300 females (12 h deprived) were released at the center of each LME starting at 1800 to 0700 (13 h) on the next day.

The mean number of mosquitoes collected with the different trapping methodologies (mean trap catches) were calculated and compared for each field site, separately. For the cage trials, data were presented as the mean proportion of mosquitoes captured for a given trap and CO_2 source.

Greece

The standard miniature CDC light trap (Model 512, John Hock) was used for capturing adult sand flies and was deployed in the field as described above. Three different combinations/configurations of the trap were tested: CDC light trap alone, CDC light trap-TDA, and CDC light trap-dry ice. The three trap combinations were positioned in three different locations within each animal farm, ~50 m apart from each other and rotated through each location for three consecutive days to reduce sampling bias. Each day trapping commenced at 1800 until 0800 on the next day (14 h).

All collected specimens were killed by holding at −20°C for 1 h and were subsequently counted and morphologically identified to species level (Lewis 1982; Leger et al. 1986a,b). Catches >200 specimens per light trap were identified using a random sample of 200 sand flies, so that total species numbers could be extrapolated to counts for the entire sample. The mean number of sand flies collected with the three trapping methodologies were compared using one-way analysis of variance with post hoc tests to determine whether there was any significance in the total number of sand flies collected and the total number of sand fly species (trap methodology = the main effect; number of collected sand flies/species = the dependent variable).

Results and Discussion

Thailand

A total 3,231 and 9,567 mosquitoes were sampled in Mae Sot and KPP Muang Districts, respectively. The following species were identified: *Anopheles aconitus, Anopheles annularis, Anopheles barbirostris, Anopheles culicifacies, An. dirus, Anopheles dravidicus, Anopheles harrisoni, Anopheles jamesii, Anopheles kochi, Anopheles maculatus, Anopheles minimus Theobald, Anopheles notanandi, Anopheles peditaeniatus, Anopheles philippinensis, Anopheles sawadwongporni Rattanarithikul & Green, Anopheles tessellatus, Anopheles vagus, Anopheles varuna, Anopheles willmori, Ae. aegypti, Aedes albopictus, Aedes lineatopennis, Aedes vexans, Armigeres subalbatus, Culex bitaeniorhynchus, Culex brevipalpis, Culex fuscocephala, Culex gelidus, Culex nigropunctatus, Culex quinquefasciatus, Culex tritaeniorhynchus Giles, Culex vishnui Theobald, Coquillettidia crassipes, Downsiomyia* sp., *Finlaya flavipennis, Mansonia indiana,* and *Uranotaenia* sp.. The most dominant species were *Cx. vishnui* > *An. minimus* > *An. sawadwongporni* > *Cx. tritaeniorhynchus*.

In Mae Sot district, mean trap catches were calculated for *Anopheline* species only (pooled together) because all other species yielded low and inconsistent numbers across traps (Fig. 2A). Similarly, in KPP Muang district, mean trap catches were calculated for all mosquito species pooled together (Fig. 2B). In both study sites, only the TDA treatment (CDC light trap + TDA) and the dry ice treatment (CDC light trap + dry ice) had mean trap catches significantly greater than the light trap alone. Also, the TDA treatment was the only treatment with no significant difference from the dry ice-treatment in mean trap catches. A probable explanation for the low performance of the MEC generators could be that the oxalic acid ring was not entirely consumed, which is required to produce the CO_2 rate claimed by the manufacturer. A recent study of the

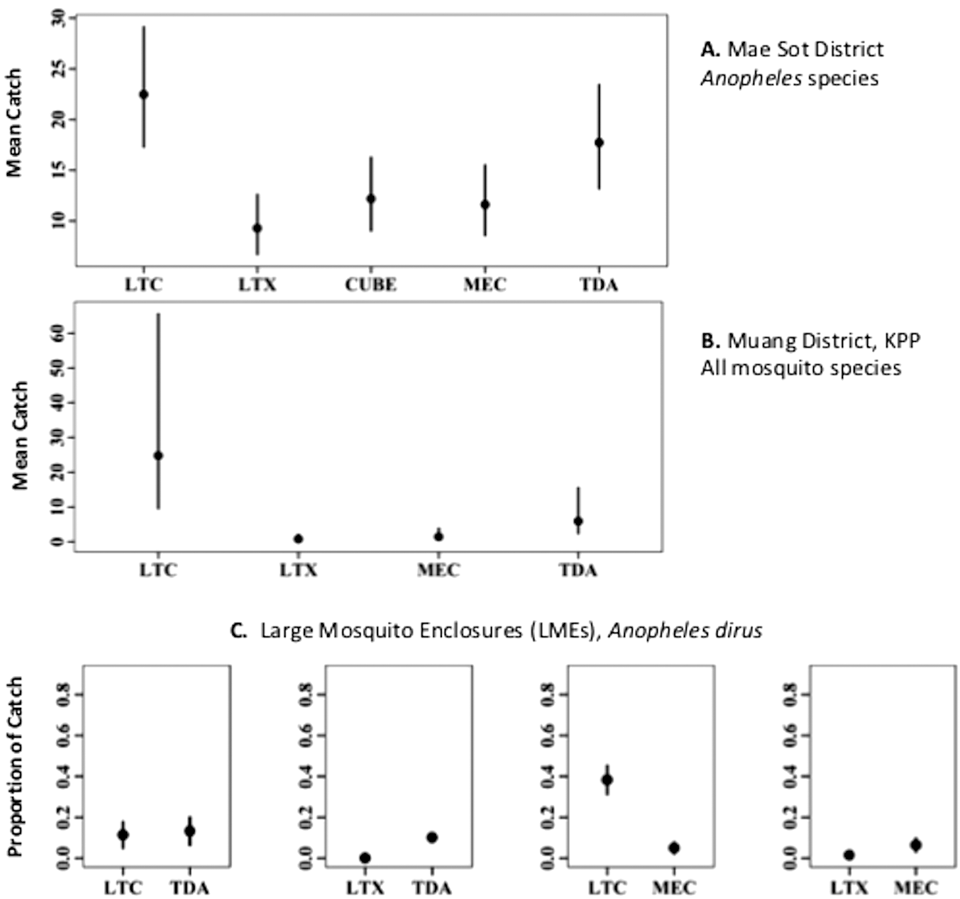

Fig. 2. Mean trap catches (±SE) of *Anopheles* mosquitoes from Mae Sot District (A) and all mosquitoes from Muang District, KPP. (B) Collected in CDC light traps baited with dry ice (LTC), no CO_2 source (LTX), CO_2 produce by CUBE generator (CUBE), CO_2 produced by MEC generator (MEC), and CO_2 produce by TDA generator (TDA). (C) Represents the mean proportion of a standard number of *Anopheles dirus* mosquitoes collected in each trap/generator combinations during head to head trials in LMEs.

same generators concluded that the CO_2 release rate was 10% of what the manufacturer claimed (Harwood et al. 2014). Variation in CO_2 release rate can significantly alter mosquito capture and often the CO_2 release rate is positively correlated with the numbers of captured adult female mosquitoes (McPhatter and Gerry 2017). The nonconsistent CO_2 release rate produced by the MEC generator may explain the lower mosquito capture performance compared to the dry ice source and the TDA generator. The CUBE generators exhibited multiple failures during the field trials, most commonly due to failure to start and/or run for the entire trapping period, and therefore data analysis could not be completed for the Muang district study site.

Similar to the field trials, the TDA treatment performed as well as the dry ice treatment in semifield studies (Fig. 2C). The dry ice treatment outperformed the MEC treatment and MEC and TDA treatments outperformed the light trap alone treatment.

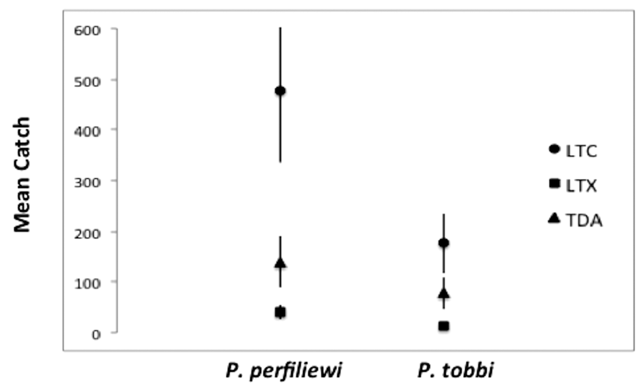

Fig. 3. Mean trap catches (±SE) of *P. perfiliewi* and *P. tobbi* from Thessaloniki, Greece collected in CDC light traps baited with dry ice (LTC), no CO_2 source (LTX) and CO_2 produce by TDA generator (TDA).

Greece

A total of 8,329 sand flies were sampled during the experimentation. By far the most prevalent sand fly species were *Phlebotomus perfiliewi* Parrot followed by *Phlebotomus tobbi* Adler and Theodor. A few *Phlebotomus simici* Nitzulescu sand flies were also collected, although in very low numbers and inconsistently across traps or trapping periods. Dry ice-baited CDC light traps collected the most sand flies, followed by TDA-baited traps and, finally by un-baited light traps (Fig. 3). The TDA-baited trap performed well in collecting high numbers of all sand fly species present in the study sites and in the case of *P. tobbi* no significant difference was observed in trap catches between dry ice and TDA-baited traps.

Both the CUBE and MEC generators had performance issues, which resulted in their failure to operate properly and consistently though out our trials and therefore were not used in the sand fly study in Greece.

Conclusions

The CUBE and MEC generators had performance issues, which prevented their consistent operation throughout our trials, and thus conclusions could not be reached with certainty regarding their overall collection efficiencies. The TDA generator, based on both collection efficiency as well as efficiency of use in the field, was the best performing CO_2 generator and for most cases was as attractive as the dry ice-baited treatments. The generator was able to attract a large variety of both nuisance and pathogen transmitting mosquito species belonging to six genera, as well as, three Phlebotomine sand fly species that are known vectors of leishmaniasis. Even though similar CO_2 generating systems have been tested on mosquitoes (Burkett-Cadena 2015), to the best of our knowledge, this is the first report on the suitability of these systems in attracting sand flies. Even though light-only baited traps are been used effectively for sand fly sampling, the addition of CO_2 source significantly increases the trap catch. This is most probably attributed to the long-range attractant effect of the CO_2 compared to light-only which is known to attract blood seeking insects from a much closer range (Alten et al. 2015). This increased sampling efficiency observed in traps baited with CO_2 indicates that this approach would be most appropriate for seasonal activity/patterns studies or the detection of sand fly-borne pathogens.

Our study further supports the suitability of CO_2 generated from carbonates and acids for mosquito as well as sand fly surveillance. Dry ice still remains the superior mosquito attractant, however, in cases where dry ice is not available the TDA CO_2 generator can function as an effective and accessible alternative.

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