Acta Tropica 149 (2015) 254–261

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Carbon dioxide generated from carbonates and acids for sampling blood-feeding arthropods

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ARTICLE INFO

Article history:
Received 7 May 2015
Received in revised form 16 June 2015
Accepted 17 June 2015
Available online 20 June 2015

Keywords:
Carbon dioxide
Mosquito
Blackfly
Surveillance
Arthropod vectors

ABSTRACT

Carbon dioxide (CO_2) is utilized to attract mosquitoes and other blood-feeding arthropods to traps around the world. Commercial forms of CO_2 (*e.g.*, dry ice and compressed gas) are often unavailable or extremely expensive in developing nations, where vector surveillance is essential to make life-saving decisions. We developed and tested inexpensive and reproducible methods of CO_2 production from the combination of acids and carbonates, ranging from very basic (crushed seashells and vinegar) to relatively elaborate (a device that controls the timing of the acid-carbonate reaction and extends the reaction over several hours). When utilized with mosquito traps in Florida, USA and black fly traps in Region des Cascades, Burkina Faso, these carbonate-acid CO_2 sources attracted significantly greater numbers of both vector groups, than did unbaited traps. CO_2 was generated for more than four hours at levels sufficient to attract vectors over the entire period. The utility of this simple methodology in developing nations should be further evaluated.

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1. Introduction

The importance of carbon dioxide (CO_2) in host location by blood-feeding arthropods cannot be overstated. Diverse blood-feeding arthropod groups, from ticks (Arachnida: Ixodida) (Norval et al., 1989), to insects, including Diptera (Guerenstein and Hildebrand, 2008), Hemiptera (Barrozo and Lazzari, 2004) and Siphonaptera (Osbrink and Rust, 1985), demonstrate positive taxis in response to a CO_2 source (chemotaxis) for host location. Experimental evidence indicates that CO_2 (and odors from host animals) guide host location of blood-feeding Diptera over longer ranges than other host-associated cues, such as heat, moisture and visual cues (color, size, shape, and movement) (Mboera and Takken, 1997).

In the United States and other developed nations, the acquisition of CO_2 for surveillance of blood-feeding arthropods rarely poses a serious challenge. CO_2 is readily available as compressed "bottled" gas or as solid "dry ice". Compressed gas is often dispensed from tanks using gas regulators that control the flow of CO_2, but add to the cost of traps, as a regulator is required for each CO_2 tank in use. Dry ice is routinely used in concert with traps in developed nations, as a blood-feeding arthropod attractant. In this context dry ice is often placed inside an insulated container, with one or more small holes (sometimes fitted with tubing) that allow the escape of subliming CO_2 gas (Silver 2008). While commercial forms of CO_2 are effective they are often difficult to acquire in developing nations. Even when available, the expense of bottled gas and the rapid sublimation of the dry ice in tropical environments reduce their utility. The need for accessible CO_2 for arthropod-borne disease surveillance in developing nations has lead to research and development of "alternative" sources of CO_2 to enhance the abundance and/or diversity of blood-feeding arthropods attracted to trapping systems.

A number of chemical and biological methods of CO_2 production have been explored by researchers for augmenting trap captures of blood-feeding arthropods. The production of CO_2 as a byproduct of microbial metabolism (Guerenstein et al., 1995) produces CO_2 (and other volatile metabolites) attractive to mosquitoes (Saitoh

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Abbreviation: CDC, centers for disease control and prevention.

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http://dx.doi.org/10.1016/j.actatropica.2015.06.019
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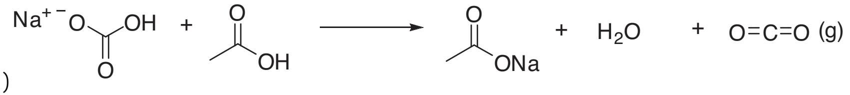
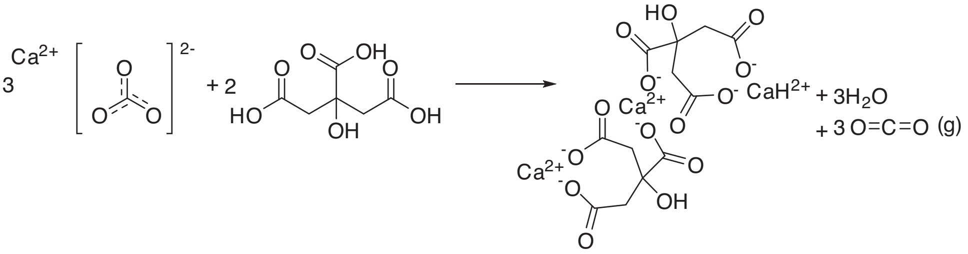
et al., 2004; Smallegange et al., 2010) the kissing bug *Triatoma infestans* (Guerenstein et al., 1995) and blackflies (Rodríguez-Pérez et al., 2013; Toé et al., 2014).

Chemical production of CO_2 is possible by a number of routes. Electrolysis of oxalic acid produces CO_2, however traps baited with electrolyzed oxalic acid had only slightly larger numbers of mosquitoes than did unbaited traps, far fewer than traps baited with compressed gas or yeast-generated CO_2 (Harwood et al., 2014). Commercial sachets for CO_2 production from proprietary dry ingredients (John W. Hock, Florida, USA) showed promise in olfactometer and large cage bioassays, attracting greater numbers of mosquitoes than 5 mL/min CO_2 from compressed gas cylinders (Kline et al., 2006). In field tests, however, dry ice baited traps collected roughly six times more mosquitoes than did traps baited with the sachets (Xue et al., 2008).

Another chemical means of CO_2 production that has received relatively little attention is the combination of carbonates (a salt of carbonic acids $[CO_3]$ or bicarbonates ($[H_2CO_3]$) and weak acids (acetic acid and citric acid, *e.g.*) in aqueous solution. Sodium bicarbonate reacts with acetic acid to produce sodium acetate, water, and CO_2 (Eq. (1)). Calcium carbonate reacts with citric acid to produce calcium citrate, water and CO_2 (Eq. (2)). In field tests, tick traps baited with 20% lactic acid and calcium carbonate captured 1606 *Amblyomma* spp. ticks (Cançado et al., 2008) and overall tick numbers not different from traps baited with dry ice (Guedes et al., 2012). While these studies with ticks have demonstrated the potential of carbonates and acids for attracting blood-feeding arthropods, this methodology has not been fully explored with other groups of blood-feeding arthropods, particularly Diptera.


(1)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In the current study we explored a method of producing CO_2 that has not been fully explored, the combining of carbonates (as salt of carbonic acids or as bicarbonates) and weak acids (acetic acid and citric acid) in aqueous solution to produce carbon dioxide. The effectiveness of the “carbonate-acid” CO_2 for attracting mosquitoes to light traps was tested at three field locations in Florida, USA and for attracting African blackfly vectors of river blindness to Esperanza window traps in Burkina Faso. To further the utility of the carbonate-acid system, an inexpensive programmable device was developed that initiates the reaction at any desired time.

2. Materials and methods

2.1. Reagents and raw materials

Readily available compounds, such as household supplies, construction materials or culinary ingredients were investigated, as they would have the greatest likelihood of being available in developing nations. Carbonates included calcium carbonate ($CaCO_3$) and sodium bicarbonate ($NaHCO_3$). Calcium carbonate was used in the form of seashells. Two varieties of seashells were used, coquina, a sedimentary rock composed of shell fragments, and crushed shell gravel, a mined subsurface deposit of marine shells from prehistoric seas (Mitterer 1974). Food-grade sodium bicarbonate was purchased from local supermarkets (baking soda, bread soda, cooking soda or bicarbonate of soda). The two acids used were acetic acid ($C_2H_4O_2$) and citric acid ($C_6H_8O_7$). Acetic acid was purchased from local supermarkets (5%; distilled white vinegar), while citric acid (crystalline) was purchased from a food supply vendor and then prepared as 5% or 10% aqueous solution (w/v).

2.2. Field evaluations

Five separate field experiments were conducted to test the effectiveness of carbonate-acids for attracting blood-feeding arthropods to traps, compared to traps with and without other sources of carbon dioxide. In Florida, USA, four field trials were conducted to evaluate relative effectiveness of carbonate-acid generated CO_2 to attract mosquitoes, using CDC miniature light traps (Model 2836BQ, BioQuip Products, Inc., Rancho Dominguez, CA). In Burkina Faso, a single field trial was conducted to evaluate the relative effectiveness of carbonate-acid generated CO_2 to attract blackflies, using the Esperanza window trap (Toé et al., 2014). Experiments utilized either of two forms of carbonate, calcium carbonate (shells) or sodium bicarbonate (baking soda), and either of two forms of acid, acetic acid (vinegar) or citric acid, in aqueous solution.

Experiment 1. The first attempt to assess the feasibility of combining calcium carbonate with weak acids to generate CO_2 for baiting traps utilized relatively small amounts of reagents and simple CO_2 production vessel. This experiment compared CDC miniature light traps baited with calcium carbonate (shell gravel) plus acid (vinegar) with unbaited traps. Light bulbs were removed from the traps, in order to minimize the confounding effect of light. Traps were hung from vegetation, so that the trap intake was 1.5 m above ground. The traps were separated by a short distance (10 m) so that they would compete for host-seeking females over this short range. Shell gravel was washed, dried and sifted using standard testing sieves (Fisher Scientific) to obtain relatively uniform particles (0.85–1.70 mm dia.), prior to use in field studies. Approximately 30 min prior to dusk (2000 h) shell gravel (100 g) and acetic acid (1.75 L; 5% aqueous) were combined in a 2 L reaction vessel (plastic bottle). The cap of the bottle was fitted with 3.0 mm i.d. hose barb and PVC tubing to deliver gaseous products of the reaction to the vicinity of the trap intake. Traps were operated from dusk until the following morning (0800 h) on seven occasions, during June and July. Bait position was alternated each trapping period. Mosquitoes were identified using morphological keys (Darsie and Ward 2005). The field site was a county greenway along the Hillsborough River in the city of Temple Terrace, Hillsborough County, FL, USA. The habitat was a lowland hardwood forest dominated by live oak, *Quercus virginiana*, water oak, *Quercus nigra* and cabbage palm, *Sabal palmetto*.

Experiment 2. A second experiment evaluating the use of calcium carbonate (shell gravel) and acid (citric acid) to attract

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