New Tool for Mosquito Surveillance: Development of a Chemical CO_2 Generation Device

by Frank H Cornine III and John D Prohaska

In this article we describe our recent work on the development and field demonstration of a new type of carbon dioxide baiting source for mosquito surveillance. Everyone involved in mosquito control is aware of the close relationship between carbon dioxide and mosquito attraction. Mosquitoes use carbon dioxide plumes to find host animals for blood feeding. It is well documented that the use of carbon dioxide greatly enhances mosquito collection using the standard Center for Disease Control (CDC) trap. Effective mosquito and disease vector surveillance depends on collecting a sufficient number of specimens to assess public health risks. The overall goal is to develop more efficient baiting methods and devices that increase collection efficiency, improving the accuracy of public health risk assessments.

The suspected link between carbon dioxide and mosquito attraction dates back to perhaps sometime in the late 19th century, but it wasn't until 1922 when Willem Rudolfs first showed that CO_2 affects mosquito behavior (Rudolfs 1922). Since then, there have been many studies documenting the enhanced collection efficiency when using carbon dioxide in mosquito trapping and its influence on mosquito feeding behavior (Gillies 1980). There are other chemicals, such as lactic acid and octenol that are attractive to mosquitoes. When combined with carbon dioxide, these act to effectively attract mosquitoes at a distance. But given all the science regarding the role of CO_2 as an attractant, it is clear from a practical point of view that carbon dioxide when used with a CDC trap is an effective method to sample mosquito populations.

In the United States and Canada there are hundreds of public mosquito control districts that manage the health risk from mosquito populations, yet many of them do not use carbon dioxide as an attractant during their collections. Of course the needs of each district vary depending on its geographical and human population characteristics, so one would not expect every district to have an extensive mosquito population monitoring program. However, it is somewhat surprising that not more of the mosquito collection programs use carbon dioxide as an attractant since the benefit of using carbon dioxide during mosquito surveillance is well documented. Given this, one is tempted to ask why? Does carbon dioxide cost too much? Is the process too complicated? Are the associated safety issues an impediment?

In general for mosquito surveillance, one wants a consistent and controllable CO_2 flow rate between 200-700 ml/min. There are several methods to generate carbon dioxide for mosquito baiting. These include dry ice (solid CO_2), compressed gas cylinders, and hydrocarbon fuel combustion. Dry ice is a

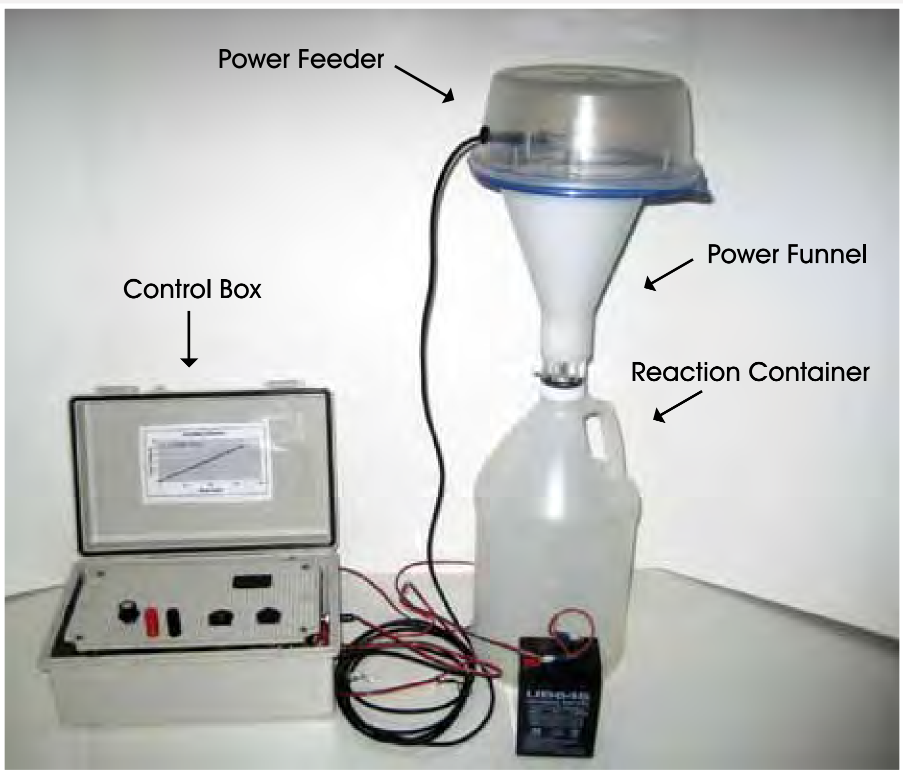


Figure 1: The Blackstone Photonics CO_2 baiting source.

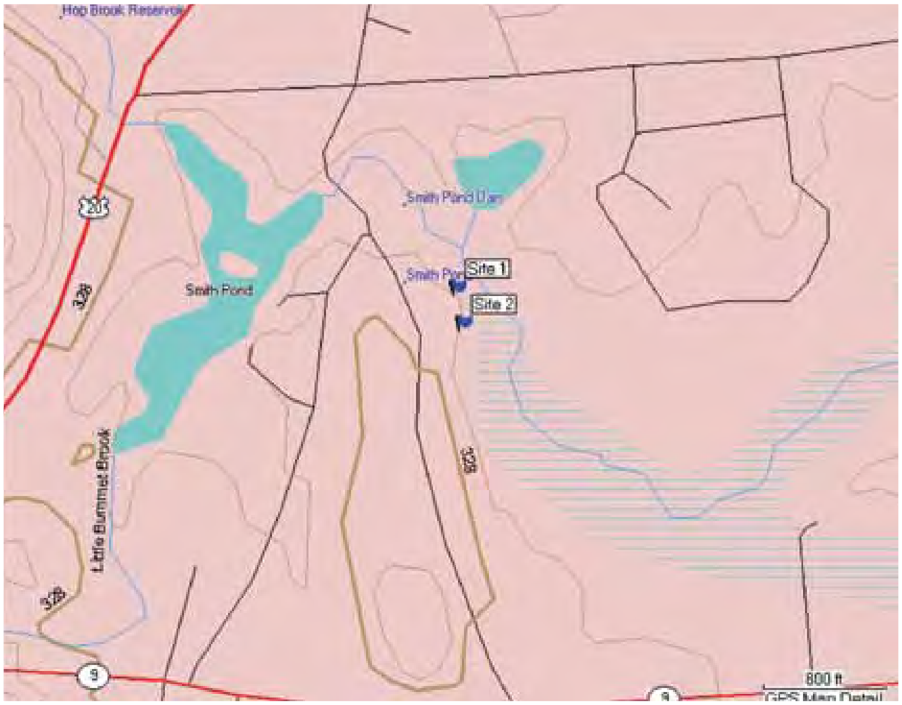


Figure 2: Mosquito monitoring test sites in Northborough, MA.

very cold material (-109°F) and requires specialized handling, refrigeration, thermoses, and insulated gloves. It sublimes at a rate of 5-10 lbs per 24 hours in a typical thermos container. This sublimation rate corresponds to a CO_2 flow of 800-1600 ml/min. The exact flow rate is difficult to control because it depends on thermal insulation factors, ambient weather conditions, and the amount of dry ice used. Weight is not much of an issue since common thermoses weigh only 1-2 pounds, but the storage and handling of CO_2 are challenges for this method. Compressed gas cylinders provide very accurate gas flow control and can readily satisfy mosquito monitoring requirements, but they are relatively heavy. A full 5 lbs gas cylinder weighs either 17.5 or 12.5 lbs for a steel or aluminum cylinder, respectively. The pressure regulator weighs another 2-4 lbs. In addition they present a safety hazard. When mishandled they can explode and become a projectile that can be quite destructive. Hydrocarbon fuel, such as propane, can be used to generate CO_2 through combustion. The primary issue with any fuel is that it creates substantial amount of heat associated with combustion which presents a fire hazard for unattended collection stations.

Blackstone Photonics of Millbury, Massachusetts has been developing a new carbon dioxide source for mosquito collecting. It is based on a novel chemical reactor design and specially modified alkali bicarbonate chemistry. We have applied our experience in meso-scale materials developed for optical chemical sensing to develop this technology. The chemical reactor is made of light weight plastic and is electronically controlled, powered by a battery; see Figure 1. Including the reaction raw materials the entire unit weighs less than 7 lbs, making it very convenient to transport to remote monitoring sites. The chemistry is ecologically friendly and is composed of items one might find in a kitchen cupboard, presenting no hazard to the operator or the environment. The reactor allows us to electronically control the rate of reaction and therefore the carbon dioxide flow rate between 0-800 ml/min. The device is easy to set-up and use. Set-up consists of pouring a dry powder into one container and mixing water with another powder in a second container which can be done before heading into the field. The unit can also be programmed to turn on and off at a specific time.

In early 2010 Blackstone Photonics approached the Central Massachusetts Mosquito Control Project (CMMCP) in Northborough, MA for advice in mosquito collection techniques and began a collaboration which bore the results discussed in this article. Together we planned a study comparing the collection efficiency of the new Blackstone CO_2 source with a standard gas cylinder source. A convenient site was identified for the study that we thought would yield good numbers of various species (predominately *Coquillettidia perturbans*). The site was located in Northborough, MA near local wetlands; see Figure 2. Two collection stations were used; one with the standard gas cylinder and the other with Blackstone source. They were separated by about 100 ft to minimize competition between the traps and switched between trap nights. CO_2 flow rates were adjusted to 250 ml/min. A collection bottle rotator (model 1512, John W Hock Co) with eight containers was used to gather information about how the collections proceeded through the night. Each trap was mounted on a 4 x 4 inch post; see Figures 3 and 4. Collection began each day three hours before sunset and the time interval for each collection cup was 90 minutes. After each night of collections we would bring the specimens and equipment back

Figure 3: Standard gas cylinder baited trap setup in the field.

Figure 4: Blackstone CO_2 generation source baited trap setup in the field.

Figure 5: Retrieving mosquito specimens from both CO_2 traps.

to the shop to identify and count mosquitoes; see Figures 5 – 7. The Blackstone baiting source would be prepared sometime during the day of the collection and would take only a few minutes. The collection period was about a month and a half from June 23 to August 5, 2010. Over this time we were able to obtain nine good collections for comparisons. During the study the Blackstone CO_2 source was exposed to a variety of weather conditions ranging from hot and humid New England summer conditions to down pours from severe thunder storms. The unit performed quite well in all conditions without any significant problems.

Based on our results we can make a number of observations and comments. Overall, we see that there is a significant fluctuation in the number of mosquitoes collected from night to night and also from CO_2 source to source; see Figures 8 and 9. Night-to-night fluctuations are expected given variations in the evening temperature, wind speed, and humidity. In addition we see a general trend of the total mosquito count decreasing as the season progresses, which is also expected given the extended drought that coincided with collections; see Figure 8. This general trend essentially forced us to stop the study prematurely. In examining source to source variability for a given night's collection we observed, in some

Figure 6: Mosquito samples from a collection bottle ready to be identified.

cases, a substantial variation and in others virtually none. If we look at the cases more closely we see that two trials show significantly greater counts for the gas cylinder (numbers 3 & 6) and two other trials show significantly greater counts for the Blackstone source (numbers 2 & 4). For the remaining trials, the collection numbers are essentially equal; see Figure 8.

Since the total number of collections represents a relatively small sample, it is difficult to determine precisely the origin of (and to assess the statistical significance) the variation we observe. The origin of the variation may be instrumental and or environmental in nature. In an effort to minimize any instrumentally induced error, we have performed extensive calibration testing of the Blackstone CO_2 source with a reference gas cylinder. These calibration tests show good agreement between the two sources. Local environmental variations could be another possible explanation. The local geography of the sites varied somewhat which could lead to microclimate differences that affected the mosquito counts for those specific cases. Either way, on average these variations may not be significant. The total number of mosquitoes collected over the entire period using the standard gas cylinder and the Blackstone source as bait was 408 and 564, respectively.

When examining the data for the number of mosquitoes collected in 90 minute intervals after sunset for two collection nights (7/8, 7/20) we made a number of interesting observations; see Figure 9. First, traps using both CO_2 sources exhibited a similar time collection response. We also observed that the majority of mosquitoes were collected during the first 180 minutes after sunset for both carbon dioxide sources with little activity outside this period and that there is a peak occurring in the first 90 minutes after sunset. These observations are consistent with previous host-seeking activity studies for similar mosquito species (Schmidt 2003).

In conclusion, Blackstone Photonics and the CMMCP compared the effectiveness of a new carbon dioxide source compared with a standard gas cylinder using a CDC mosquito trap. The number

Figure 7: Counting and identifying mosquito specimens from trap collection.

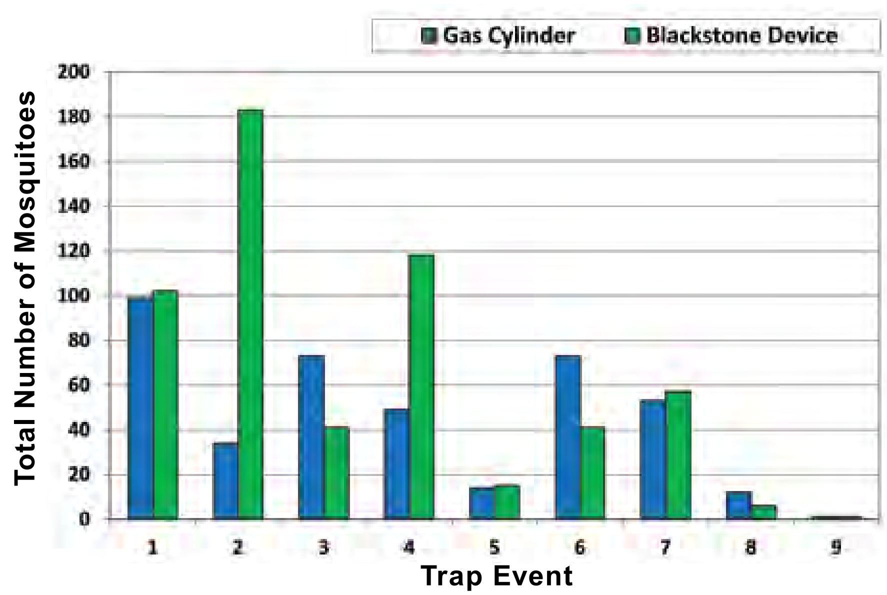


Figure 8: Collection night mosquito totals for both gas cylinder and Blackstone CO_2 baited traps.

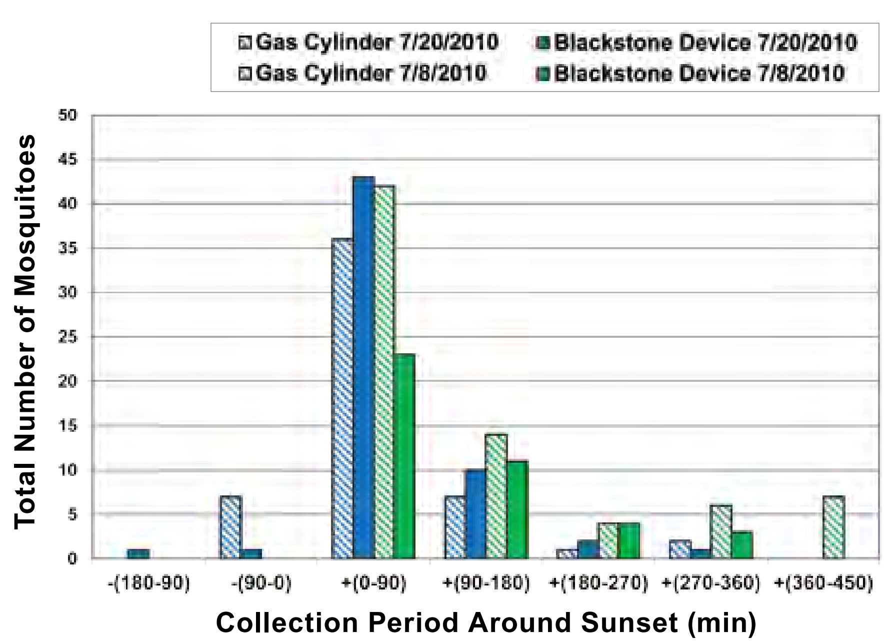


Figure 9: Mosquito collection numbers in 90 minute intervals around sunset for select trap nights of both gas cylinder and Blackstone CO_2 baited traps.

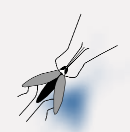
of mosquitoes collected during this study show a good correlation between the two gas sources indicating the Blackstone generator showed significant promise as a potential new carbon dioxide source for field mosquito surveillance. The new carbon dioxide source was exposed to a range of weather conditions and continued to function without any major problems. The new Blackstone Photonics carbon dioxide source offers significant benefits over existing gas sources for mosquito baiting. These advantages include; light-weight, contains environmentally friendly materials, gas flow is controlled electronically, and is convenient and safe to use. It is our belief that one day this new carbon dioxide source will become an effective tool augmenting mosquito surveillance programs. Using this CO_2 source would make it easier for some mosquito control districts to employ carbon dioxide baiting as part of their standard surveillance procedures and improve their ability to make accurate risk assessment of public health risks from mosquitoes.

REFERENCES CITED

Gillies, MT. 1980. The role of carbon dioxide in host-finding by mosquitoes (Diptera: Culicidae): a review. Bull Entomol Res 70: 525–532.

Rudolfs, W. 1922. Chemotropism of mosquitoes. Bull NJ Agric Exp Stn No 367.

Schmidt, RF. 2003. Relationship of landing count observations to the time of sunset. Wing Beats 14 (3): 12-15.



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