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Evaluation of granular carbon dioxide sachets for use in combination with CDC and EVS light traps for collecting mosquitoes.

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Dr. Cameron Webb PhD. and Assoc. Prof. Richard C. Russell MSc. PhD.
Department of Medical Entomology, University of Sydney & ICPMR, Westmead Hospital
Level 3, ICPMR, Westmead Hospital, Westmead NSW 2145
http://www.medent.usyd.edu.au | cameronw@icpmr.wsahs.nsw.gov.au

Introduction

Carbon dioxide is the most common attractant used in adult mosquito traps for pest and vector monitoring. The production of carbon dioxide from novel sources (eg. granules enclosed in sachets) holds great potential for use in ecological and public health investigations where mosquito sampling is required in remote locations and/or where the supply of dry ice or cylinder carbon dioxide is limited.

ICA has developed granules that release carbon dioxide and can be packaged in sachets for use with mosquito traps. The sachets developed for mosquito surveillance purposes are designed to release over 70% of their potential carbon dioxide output within 24 hours. In laboratory trials, the sachets have an average rate of release about 1-3 ml/min that can be sustained over 24 hours (pers. comm. Joel Tenney, ICA).

The aim of this investigation was to evaluate the suitability of the granule sachets as an alternative to dry ice as a source of carbon dioxide for use with adult mosquito traps by comparing the range and quantity of species collected using the two sources of carbon dioxide.

Methods

The study site was a forested area close to estuarine and freshwater wetlands at Homebush Bay, Sydney. The site provided an ideal location to evaluate the response of a range of mosquitoes to the sachets as over 30 mosquito species have

been identified from this region including major pest and vector species such as *Ochlerotatus vigilax*, *Coquillettidia linealis* and *Culex annulirostris*.

A 4x4 latin-square design was used with a total of 16 traps used per night over four consecutive nights from Tuesday 16 March through to Friday 19 March 2004. Encephalitis Vector Surveillance (EVS) miniature light traps and Centers for Disease Control (CDC) miniature light traps baited with either dry ice (approximately 500g per night) or the sachets (a fresh sachet per trap per night). Traps were set 2-3 hours before sunset and collected the following morning (after approximately 16-18hrs). Collections were returned to the laboratory for identification and sorting.

The mean number of mosquitoes collected by each trap was log (n+1) transformed and ANOVA was used to determine any significant difference in the number of mosquito species, the total number of mosquitoes collected and the total number of the most common species of mosquito by the two trap types baited with either dry-ice or sachets.

Results and discussion

Climatic conditions remained consistent over the four nights with the mean maximum temperature 25.8^{o}C $\pm$ 1.6^{o}C, mean minimum temperature 19.3^{o}C $\pm$ 1.3^{o}C, mean relative humidity was 64.1 $\pm$ 2.1% and there was less than 1mm of rain recorded.

A total of 18 mosquito species was collected over the four days with the most common species *Cq. linealis*, *Cx. annulirostris*, *Ochlerotatus alternans* and *Oc. vigilax* (Table 1). Traps baited with sachets collected, on average, 4.7 % (range 0 - 20%) for CDC traps and 7.4% (range 0 - 55%) for EVS traps of the total numbers collected by those traps baited with dry ice.

Of the 18 species collected there were less than 3 individuals of 7 of the species and these were only from traps baited with dry ice. No species were collected only from traps baited with the sachets. Traps baited with dry-ice collected significantly more species than traps baited with the sachets but there was no significant difference in the number of species collected by the EVS and CDC traps baited with the sachets (Table 2).

Traps baited with dry ice collected significantly more mean total mosquitoes, mean total *Culex* spp., mean total *Ochlerotatus* spp., *Cq. linealis*, *Cx. annulirostris* and *Oc. vigilax* than traps baited with the sachets (Table 3). For traps baited with the sachets, there were significantly more *Oc. vigilax* collected in CDC traps than EVS traps but was no significant difference in the mean total mosquitoes, mean total *Culex* spp., mean total *Ochlerotatus* spp, *Cq. linealis* or *Cx. annulirostris* collected in the CDC or EVS traps.

Table 1. The total number of mosquitoes collected by CDC and EVS traps baited with either dry ice or sachets over four nights at Homebush Bay.

Mosquito species	CDC+ DRY ICE	CDC+ SACHETS	EVS+ DRY ICE	EVS+ SACHETS
Anopheles annulipes s.l.	82	3	128	1
Coquillettidia linealis	434	31	652	34
Culex annulirostris	1 145	81	1 515	62
Culex australicus	17	1	43	7
Culex molestus	7	0	39	3
Culex quinquefasciatus	12	1	29	2
Culex sitiens	110	6	235	33
Mansonia uniformis	1	0	0	0
Ochlerotatus alboannulatus	1	0	2	0
Ochlerotatus alternans	326	53	486	85
Ochlerotatus camptorhynchus	1	0	0	0
Ochlerotatus multiplex	1	0	0	0
Ochlerotatus notoscriptus	20	4	20	11
Ochlerotatus procax	9	0	13	0
Ochlerotatus quasirubrithorax	1	0	0	0
Ochlerotatus vigilax	7 298	713	6 542	422
Verrallina funerea	2	0	0	0
Verrallina Marks #52	2	0	0	0
TOTAL	9 469	893	9 704	660

Table 2. The mean number (± SE) of mosquito species collected by CDC and EVS baited with either dry ice or sachets over four nights at Homebush Bay.

CDC+ DRY ICE	CDC+ SACHETS	EVS+ DRY ICE	EVS+ SACHETS
8.4 ± 0.3 (6-11) A[1]	4.4 ± 0.3 (3-8) B	8.4 ± 0.5 (4-12) A	5.3 ± 0.4 (2-8) B

[1] Means in same row followed by the same letter are not significantly different (P<0.05)

Table 3. Mean number (± SE) of total mosquitoes, selected mosquito species and mosquito genera collected in CDC and EVS traps baited with dry ice or sachets over four nights at Homebush Bay.

	CDC+ DRY ICE	CDC+ SACHETS	EVS+ DRY ICE	EVS+ SACHETS
Coquillettidia linealis	27.1 ± 3.1 B[1]	1.9 ± 0.4 C	40.8 ± 5.9 A	2.1 ± 0.5 C
Culex annulirostris	71.6 ± 14.5 A	5.1 ± 1.1 B	94.7 ± 13.0 A	3.9 ± 0.8 B
Ochlerotatus alternans	20.4 ± 3.4 A	3.3 ± 0.5 B	30.4 ± 7.5 A	5.3 ± 1.2 B
Ochlerotatus vigilax	456.1 ± 85.2 A	44.6 ± 7.4 B	408.9 ± 57.3 A	26.9 ± 5.4 C
Total *Ochlerotatus* spp.	478.6 ± 84.6 A	48.1 ± 7.4 B	441.4 ± 62.5 A	32.4 6.4 B
Total *Culex* spp.	80.7 ± 14.7 A	5.6 ± 1.1 B	116.3 ± 15.6 A	6.7 ± 1.0 B
Total mosquitoes	591.8 ± 100.1 A	55.8 ± 8.0 B	606.5 ± 71.5 A	41.3 ± 7.2 B

[1] Means in same row followed by the same letter are not significantly different (P<0.05)

These results indicate that the use of the sachets as an alternative source of carbon dioxide to dry ice may not be appropriate for many mosquito or arbovirus investigations due to the reduced collections that result from the substantially lower release rate of carbon dioxide from the sachets compared with dry ice.

The traps baited with the sachets did collect a number of mosquito species, particularly the most common species, and performed equally well when used in combination with either CDC or EVS traps. EVS traps are not used without carbon dioxide to collect mosquitoes because the light source is so small but CDC traps are often used without carbon dioxide (generally where dry ice is not available) to collect mosquitoes, and for both traps, the addition of these sachets as a source of carbon dioxide is likely to increase collections of most common target species despite the fact that it is less effective than dry ice or cylinder gas.

Thus, the greatest potential for the sachets will be for use in remote locations where the supply of dry ice and/or cylinder carbon dioxide is limited. Their use with the EVS traps (specifically designed for ease of transport) for ecological investigations in locations where there is little available information on the mosquito fauna could be quite valuable, providing their limitations were understood with respect to the diversity and abundance of their collections.

Further investigation of the sachets would be interesting, particularly their use in combination with synergising agents such as Octenol and this may provide useful information for the development of mosquito monitoring strategies that maximise the

potential of the sachets to collect mosquitoes when used in combination with either CDC or EVS traps. The use of octenol with dry ice baited traps has been shown to increase the collections of some, but not all, species and the use of octenol in conjunction with the sachets with EVS or CDC traps may increase the range of species and the total number of mosquitoes collected well above that attracted by the sachets alone.

Overall, the sachets hold potential for mosquito and arbovirus monitoring under certain circumstances, and further research and field testing should generate a combination of trap type and synergising agent that maximises the effectiveness of the sachets.