THE RESPONSE OF MOSQUITOES (DIPTERA: CULICIDAE) FROM THE SYDNEY REGION OF NEW SOUTH WALES TO LIGHT TRAPS BAITED WITH CARBON DIOXIDE AND OCTENOL

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Summary

We conducted four trials in Sydney, New South Wales, Australia to compare the numbers of mosquitoes collected in Encephalitis Vector Surveillance (EVS) miniature light traps baited with carbon dioxide, carbon dioxide plus octenol or octenol alone. A total of 12127 mosquitoes belonging to 19 species was collected. For all species, more mosquitoes were collected in traps baited with carbon dioxide, with or without the addition of octenol, than with octenol alone. There were significantly more mosquitoes of most *Ochlerotatus* species collected in traps baited with carbon dioxide plus octenol than with carbon dioxide alone. The response of *Anopheles* spp. and *Culex* spp. was variable but, generally, there was no significant response to the addition of octenol to carbon dioxide baited traps.

Keywords: mosquito sampling, mosquito trap, octenol**,** *Ochlerotatus vigilax, Culex annulirostris*

INTRODUCTION

Mosquitoes associated with estuarine and freshwater wetlands of New South Wales (NSW) have the potential to cause severe nuisance biting impacts and may represent public health risks through the transmission of mosquito-borne pathogens such as Ross River virus and Barmah Forest virus (Russell 1998). In coastal areas, *Ochlerotatus vigilax* (Skuse) is the major pest and vector (disease-carrying) species and is closely associated with estuarine wetlands. Mosquitoes associated with freshwater habitats such as *Culex annulirostris* Skuse and *Coquillettidia linealis* (Skuse), as well as those associated with urban habitats such as *Culex quinquefasciatus* Say and *Ochlerotatus notoscriptus* (Skuse), are of increasing concern as a source of potential pest and public health risks (Russell 1998).

The routine sampling of pest and vector mosquitoes in NSW is commonly undertaken using miniature light traps known as Encephalitis Vector Surveillance (EVS) traps (Rohe and Fall 1979) baited with the attractant carbon dioxide. The need to collect greater numbers of mosquitoes to assist ecological investigations or for the isolation of mosquito-borne disease pathogens requires an optimisation of the attractiveness of traps to host seeking mosquitoes.

Octenol (1-octen-3-ol) is an attractant compound that can be isolated from the breath of oxen (Hall *et al.* 1984). When used in combination with carbon dioxide, octenol has increased the collection of haematophagous insects belonging to the Dipteran Families Simuliidae (Atwood and Meisch 1993), Ceratapogonidae (Kline *et al.* 1994), Tabanidae (French and Kline 1989) and Culicidae (Kline *et al.* 1991, Van Essen *et al.* 1994, Kemme *et al.* 1993, Ritchie and Kline 1995).

In southeast Queensland Kemme *et al.* (1993) found that EVS traps baited with octenol collected significantly greater numbers of *Oc. vigilax* but not significantly more *Cx. annulirostris.* However, paired trapping studies by Ritchie and Kline (1995) found that the addition of octenol to EVS and Center for Disease Control (CDC) miniature light traps resulted in the collection of significantly greater numbers of *Oc. vigilax* and *Cx. annulirostris.*

The aim of this investigation was to determine if the addition of octenol to carbon dioxide baited EVS traps resulted in significantly improved collections of mosquitoes from the Sydney region and whether octenol could be used to improve the surveillance of pest mosquitoes and mosquito borne disease pathogens in NSW.

MATERIALS AND METHODS

The study site was a mixed Eucalypt forest close to extensive estuarine and freshwater wetlands in Royal Australian Navy Armaments Depot (R.A.N.A.D.) Newington, now part of Sydney Olympic Park, Sydney, NSW. A total of 31 mosquito species has been recorded from the greater Homebush Bay region providing a representative sample of the known mosquito fauna of the Sydney basin (Webb *et al*. 2001).

Nine fixed trap sites were set on three transects running NW:SE approximately 100 m apart. Traps

were operated from one hour before dusk until two hours after dawn. Along each transect, the three traps were spaced approximately 50 m apart.

Octenol was released from a 20 mL microreaction vial fitted with an aluminium lid, neoprene septum and pipe-cleaner wick (approximately 15 mm) (Van Essen *et al*. 1994) that was attached to the EVS trap by an elastic band. Each vial contained 10 mL of octenol.

Four trials were undertaken between February and April 1999 to document the response to octenol and carbon dioxide baited EVS traps of the greatest diversity of mosquito species in the local area. Each trial was carried out over three consecutive nights using a 3 x 3 latin square design (Cochran and Cox 1957) to compare the mean number of each mosquito species collected in traps baited with either carbon dioxide (dry ice), carbon dioxide plus octenol, or octenol alone. In each trial, the trap positions were randomised and then rotated each night so that each treatment occupied a different trap position over the three nights. The number of mosquitoes per trap was log (x+1) transformed. Analysis of variance (ANOVA) was used to compare the collections. Multiple comparison tests were utilised to determine significant differences in the mean numbers of mosquitoes collected in the three trap bait configurations.

Temperature and rainfall data was obtained from the Bureau of Meteorology, Homebush (Olympic Stadium) Automated Weather Station.

Table 1. Total number of mosquito species collected in the four trials using EVS traps baited with either carbon dioxide, carbon dioxide plus octenol or octenol alone, between February and April 1999 at Sydney, NSW.

Species	**No. mosquitoes collected**			**Total**
	CO_2	**CO_2 + octenol**	**octenol**	
Anopheles annulipes s.l.	330	225	0	555
Coquillettidia linealis	24	64	0	88
Culex annulirostris	1 230	1 512	15	2 757
Culex australicus	37	6	1	44
Culex halifaxii	1	0	0	1
Culex molestus	28	24	1	53
Culex orbosiensis	10	2	0	12
Culex quinquefasciatus	63	19	2	84
Culex sitiens	203	69	8	280
Mansonia uniformis	0	1	0	1
Ochlerotatus alboannulatus	3	18	0	21
Ochlerotatus alternans	138	129	0	267
Ochlerotatus camptorhynchus	2	4	0	6
Ochlerotatus flavifrons	42	322	1	365
Ochlerotatus nr. *monocellatus*	0	1	0	1
Ochlerotatus notoscriptus	206	490	11	707
Ochlerotatus procax	144	506	4	654
Ochlerotatus vigilax	1 611	4 557	62	6 230
Ochlerotatus vittiger	0	1	0	1
Total	**4 072**	**7 950**	**105**	**12 127**

RESULTS

Overnight temperatures during the four trials varied with temperatures during Trial 4 (mean temperature 15.5 ± 1.7°C) much cooler than the first three trials (mean temperatures 23.0 ± 4.6°C, 19.5 ± 2.9°C and 21.7 ± 3.6°C respectively). The only rain that was recorded was during Trial 4 when a total of 19 mm was recorded over two of the three nights.

A total of 12127 mosquitoes belonging to 19 species was collected in the traps over the four trials (Table 1). The most abundant species were *Oc. vigilax*, *Cx. annulirostris*, *Oc. notoscriptus*, *Ochlerotatus procax* (Skuse) and *Anopheles annulipes* Walker s.l.. In all trials, there were significantly greater ($P<0.05$) numbers of mosquitoes collected in traps baited with carbon dioxide or carbon dioxide plus octenol than with octenol alone. For all *Ochlerotatus* spp., more mosquitoes were collected in traps baited with carbon dioxide plus octenol than carbon dioxide alone but for *Anopheles* spp. and *Culex* spp., similar numbers of mosquitoes were collected in traps baited with carbon dioxide and carbon dioxide plus octenol. However, fewer *Cx. quinquefasciatus* and *Culex sitiens* Wiedemann were collected in traps baited with carbon dioxide plus octenol than carbon dioxide alone.

In Trial 1, a total of 5585 mosquitoes was collected comprising 15 species. The most abundant mosquitoes were *Oc. vigilax,* making up 61% of all mosquitoes collected, followed by *Cx. annulirostris* (27.6%), *Cx. sitiens* (3.5%), *Oc. notoscriptus* (3.1%) and *Ochlerotatus alternans* (Westwood) (1.3%) (Table 2). The total number of *Ochlerotatus* spp. collected was significantly greater ($P<0.05$) in traps baited with carbon dioxide compared to octenol alone. The addition of octenol to carbon dioxide resulted in the collection of significantly greater ($P<0.05$) numbers than carbon dioxide alone. There were significantly fewer ($P<0.05$) *Cx. sitiens* collected in traps baited with carbon dioxide plus octenol compared to carbon dioxide alone but significantly more ($P<0.05$) than octenol alone. There was no significant difference ($P<0.05$) in numbers of total *Culex* spp. collected in traps baited with carbon dioxide plus octenol and carbon dioxide alone.

In Trial 2 there was a total of 1087 mosquitoes collected belonging to 14 species. The most abundant species were *Oc. vigilax* (53.5% of the total mosquitoes collected), *Cx. annulirostris* (14.1%), *An. annulipes* s.l. (10.0%), *Oc. notoscriptus* (8.8%), and *Oc. procax* (3.6%) (Table 2). There was no significant difference ($P<0.05$) in the numbers of any *Ochlerotatus* spp. or total *Ochlerotatus* spp. collected in traps baited with carbon dioxide plus octenol or octenol alone. The addition of octenol to the carbon dioxide baited traps resulted in significantly ($P<0.05$) fewer *An. annulipes* s.l. and significantly fewer ($P<0.05$) total *Culex* spp. collected in those traps compared with those baited with carbon dioxide alone.

In Trial 3, there was a total of 1641 mosquitoes collected belonging to 11 species, the most abundant being *Cx. annulirostris* (55.5%), *Oc. notoscriptus* (16.6%), *Oc. vigilax* (13.1%) and *An. annulipes* s.l. (9.3%) (Table 2). The addition of octenol to carbon dioxide did not result in significantly greater collections of any species.

In Trial 4, a total of 3824 mosquitoes belonging to 14 species was collected, the most abundant being *Oc. vigilax* (52.6%), *Oc. procax* (14.7%), *An. annulipes* s.l. (6.7%), *Ochlerotatus flavifrons* (Skuse) (9.5%) and *Oc. alternans* (5.1%) (Table 2). The addition of octenol to the carbon dioxide baited traps resulted in significantly greater number of *Oc. flavifrons* ($P<0.01$), *Oc. procax* ($P<0.01$), *Oc. vigilax* ($P<0.01$) and *Cx. annulirostris* ($P<0.01$) but not total *Ochlerotatus* spp. or total *Culex* spp.

DISCUSSION

The results confirm that octenol, when used in combination with carbon dioxide, has a synergistic effect on a number of mosquito species in the Sydney region. The attractiveness of carbon dioxide and octenol to some Australian mosquitoes has been demonstrated previously (Kemme *et al.* 1993, Van Essen *et al.* 1994). Our investigation has demonstrated that the attraction of *Oc. flavifrons*, *Oc. procax*, *Oc. vigilax* and *Cx. annulirostris* to carbon dioxide baited traps was increased with the addition of octenol during at least one of the trials. The response of tropical and subtropical populations of *Oc. procax* (Ritchie and Kline 1995), *Oc. vigilax* (Kemme *et al.* 1993, Van Essen *et al.* 1994, Ritchie and Kline 1995) and *Cx. annulirostris* (Ritchie and Kline 1995) to traps baited with carbon dioxide plus octenol has been shown previously. Results of our investigation indicate a similar response from these species in a temperate climate zone. Additionally, this was the first time the attraction of *Oc. flavifrons* to octenol has been reported with the species showing a strong positive response to octenol when used in combination with carbon dioxide.

The results of this investigation indicated that there was very little response of mosquitoes to traps baited

Table 2. Mean abundance (± SD) of mosquito species collected in the four trials using EVS traps baited with either carbon dioxide, carbon dioxide plus octenol or octenol alone, between February and April 1999 at Sydney, NSW.

Trial No.	Mosquito group	CO_2 (mean ± SD)	CO_2 + octenol (mean ± SD)	octenol (mean ± SD)
1	*Oc. vigilax*[1]	65.2 ± 39.6 a	312.7 ± 225.1 b	2.2 ± 2.7 c
	Total *Ochlerotatus* spp.[2]	76.7 ± 36.5 a	333.1 ± 237.4 b	2.6 ± 3.0 c
	Cx. annulirostris	74.4 ± 54.1 a	96.0 ± 75.8 a	0.9 ± 2.0 b
	Cx. sitiens	15.7 ± 14.4 a	5.6 ± 10.4 b	0.4 ± 0.7 c
	Total *Culex* spp.[3]	98.1 ± 63.6 a	103.0± 80.6 a	0.9± 2.0 b
2	*Oc. vigilax*	39.3 ± 29.8 a	24.8 ± 12.6 a	0.6 ±1.0 b
	Total *Ochlerotatus* spp.[4]	140.7 ± 81.7 a	96.0 ± 34.6 a	4.3 ± 6.7 b
	An. annulipes s.l.	9.8 ± 8.3 a	2.3 ± 3.4 b	0.0 ±0.0 c
	Cx. annulirostris	10.3 ± 12.5 a	6.2 ± 7.7 a	0.4 ±0.5 b
	Total *Culex* spp.[5]	52.3 ± 48.2 a	24.0 ± 19.9 b	2.3 ± 2.3 c
3	*Oc. vigilax*	9.4 ± 8.6 a	14.3 ± 10.0 a	0.1 ± 0.3 b
	Total *Ochlerotatus* spp.[6]	18.7 ± 18.2 a	36.4 ± 36.3 a	0.1 ± 0.3 b
	An. annulipes s.l.	9.7 ± 12.2 a	7.3 ± 9.0 a	0.0 ± 0.0 b
	Cx. annulirostris	46.2 ± 34.4 a	54.9 ± 41.2 a	0.2 ± 0.7 b
	Total *Culex* spp.[7]	48.0 ± 33.4 a	58.4 ± 44.1 a	0.3 ± 0.7 b
4	*Oc. alternans*	9.2 ±13.6 a[1]	12.3 ± 11.3 a	0.0 ± 0.0 b
	Oc. flavifrons	4.7 ± 5.0 a	35.7 ± 34.0 b	0.1 ± 0.3 c
	Oc. notoscriptus	3.9 ± 4.6 a	14.2 ± 20.0 a	0.0 ± 0.0 b
	Oc. procax	12.8 ± 13.5 a	49.2 ± 43.7 b	0.4 ± 1.0 c
	Oc. vigilax	65.0 ± 38.1 a	154.6 ± 105.9 b	4.0 ± 4.0 c
	Total *Ochlerotatus* spp.[8]	95.6 ± 55.6 a	268.6 ± 154.8 a	4.6 ± 4.9 b
	An. annulipes s.l.	14.4 ± 12.9 a	11.8 ± 7.1 a	0.0 ± 0.0 b
	Cx. annulirostris	5.7 ± 8.5 a	10.9 ± 5.5 b	0.1 ± 0.3 c
	Total *Culex* spp.[9]	12.8 ± 9.9 a	11.9 ± 5.9 a	0.2 ± 0.4 b

[1] Means in the same row followed by the same letter are not significantly different (P>0.05). Single factor ANOVA applied to log(x+1) transformed data.
[2] Includes *Oc. alboannulatus* (0.1%), *Oc. alternans* (1.9%), *Oc. camptorhynchus* (0.1%), *Oc. flavifrons* (0.1%), *Oc. notoscriptus* (4.6%), *Oc. procax* (1.1%) and *Oc. vigilax* (92.2%).
[3] Includes *Cx. annulirostris* (85.3%), *Cx. australicus* (0.3%), *Cx. halifaxii* (0.1%), *Cx. molestus* (0.3%), *Cx. orbostiensis* (0.6%), *Cx. quinquefasciatus* (2.6%) and *Cx. sitiens* (10.8%).
[4] Includes *Oc. alboannulatus* (0.3%), *Oc. alternans* (0.3%), *Oc. camptorhynchus* (0.1%), *Oc. notoscriptus* (13.9%), *Oc. procax* (5.5%) and *Oc. vigilax* (79.9%).
[5] Includes *Cx. annulirostris* (64.8%), *Cx. australicus* (8.9%), *Cx. molestus* (6.3%), *Cx. orbostiensis* (0.8%), *Cx. quinquefasciatus* (5.5%) and *Cx. sitiens* (13.6%).
[6] Includes *Oc.* nr. *monocellatus* (0.2%), *Oc. notoscriptus* (54.7%), *Oc. procax* (1.9%) and *Oc. vigilax* (43.2%).
[7] Includes *Cx. annulirostris* (94.5%), *Cx. australicus* (0.2%), *Cx. molestus* (1.9%), *Cx. quinquefasciatus* (1.0%) and *Cx. sitiens* (2.4%).
[8] Includes *Oc. alboannulatus* (0.5%), *Oc. alternans* (5.9%), *Oc. camptorhynchus* (0.01%), *Oc. flavifrons* (11.0%), *Oc. notoscriptus* (4.9%), *Oc. procax* (17.0%) and *Oc. vigilax* (60.7%).
[9] Includes *Cx. annulirostris* (67.3%), *Cx. australicus* (6.7%), *Cx. molestus* (6.3%), *Cx. quinquefasciatus* (6.3%) and *Cx. sitiens* (13.5%).

with octenol alone and were consistent with those of previous findings (Takken and Kline 1989, Kline *et al.* 1990, Kline *et al.* 1991, Kline and Mann 1998). The use of octenol alone in EVS traps is not effective for routine sampling of adult mosquitoes or arbovirus surveillance in NSW. Moreover, there is no advantage in using octenol and carbon dioxide in combination given the added costs and inconsistent response to octenol by different species. Carbon dioxide, therefore, is the most appropriate and cost effective attractant to use with EVS traps for mosquito surveillance programs.

Increasing the size of trap collections is of assistance for the monitoring of pest populations and early detection of arbovirus activity. The addition of octenol to the EVS traps can significantly increase collection of some species (eg. *Ochlerotatus* spp.), even at low temperatures, and its use during the early part of the mosquito season may be beneficial for the detection of arbovirus activity. The detection of virus activity in "amplifying" vectors such as floodwater *Ochlerotatus* spp. and spring active species such as *Ochlerotatus camptorhynchus* (Thomson) may be particularly important.

The addition of octenol to traps throughout of the mosquito 'season' may be beneficial for monitoring arbovirus activity in *Ochlerotatus* spp. In the Sydney region, and elsewhere in NSW, the abundance of *Ochlerotatus* spp. can be variable with population increases closely associated with rainfall and/or tidal inundation of wetlands. For *Oc. vigilax*, large populations result from flooding of estuarine wetlands by high tides and/or rainfall, but decline to relatively small populations within the following two weeks (Webb and Russell 1999). During these periods of low abundance, the addition of octenol to carbon dioxide baited EVS traps may be used to increase the quantity of mosquitoes collected and increase the likelihood of identifying arbovirus activity. However, while the addition of octenol to traps may be useful in some circumstances, it may also result in a disruption to the routine population surveillance by collecting greater numbers of *Ochlerotatus* spp. at the expense of *Culex* spp. and provide a hindrance to analysis of long term population trends.

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