Journal of the American Mosquito Control Association, 9(4):431–435, 1993

RESPONSE OF MOSQUITOES TO CARBON DIOXIDE AND 1-OCTEN-3-OL IN SOUTHEAST QUEENSLAND, AUSTRALIA

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ABSTRACT. Encephalitis vector surveillance (EVS) traps were used to study the attractant effect of CO_2 and 1-octen-3-ol (octenol) on mosquitoes at 2 different locations in southeast Queensland. Octenol alone was only slightly attractive for *Aedes vigilax*. There was a significant increase in the numbers of *Ae. vigilax* and *Aedes funereus* caught when octenol was added to CO_2, although catches of *Culex annulirostris* and *Culex sitiens* did not change significantly. The size and age compositions of *Ae. vigilax* attracted by CO_2 and by octenol were comparable. These data suggest that octenol should be considered as a supplement to CO_2-baited EVS traps for mosquito-based arbovirus surveillance in southeast Queensland.

INTRODUCTION

Epidemic polyarthritis is a predominant mosquito-borne disease of medical importance in Australia today (Kay et al. 1981, Kay and Aaskov 1989). *Culex annulirostris* Skuse and *Aedes vigilax* (Skuse) are major vectors of its etiological agent, Ross River virus (RR) (Kay and Aaskov 1989). *Culex annulirostris* breeds in fresh to slightly brackish water throughout Australia (Kay et al. 1981), whereas *Ae. vigilax* is a salt marsh mosquito restricted to coastal areas (Lee et al. 1984). Because urbanization, coupled with growing tourism, is concentrated on the coast, effective control of RR is based on surveillance and control of *Ae. vigilax*. Ultimately, RR surveillance will include virus assay of trapped mosquitoes. Because virus-infected mosquitoes are generally uncommon, it is imperative to maximize trap collections.

Currently, encephalitis vector surveillance (EVS) light traps (Rohe and Fall 1979), often supplemented with dry ice, are used to monitor adult mosquito populations. Studies by Takken and Kline (1989) and Kline et al. (1990a, 1991b) in the United States indicate that addition of 1-octen-3-ol (hereafter referred to as octenol) to CO_2 synergistically increases collections of aedine mosquitoes, most notably *Aedes taeniorhynchus* (Wied.), a Neotropical salt marsh mosquito. We present the results of a study to investigate the response of mosquitoes, in particular *Ae. vigilax*, to CO_2 and octenol in southeast Queensland.

The efficacy of a surveillance system based on virus isolates from mosquitoes would be enhanced if parous mosquitoes, having previously bloodfed, were selectively collected (Kline et al. 1990b). Body size is also related to survival and bloodfeeding success in mosquitoes, but no data about this relationship are available for *Ae. vigilax*. Nasci (1986) found that parous host-seeking *Aedes aegypti* (Linn.) females were significantly larger than the nulliparous host-seeking females. We also investigated if the addition of octenol to CO_2 attracted larger *Ae. vigilax*, with a higher proportion of parous individuals.

MATERIALS AND METHODS

Study area: Field studies were conducted from February 18 to March 17, 1993, in 2 locations adjacent to *Ae. vigilax* breeding sites in southeast Queensland. The first location, Rocky Point (42 km SE of Brisbane) is a salt marsh dominated by *Sporobolus virginicus* (Linn.) Kunth, *Sarcocornia quinqueflora* (Bunbe *ex.* Ung.-Stern) A. J. Scott, and *Avicennia marina* Vierh. The second location is near the Brisbane airport (14 km NE of Brisbane) in a grove of *Casuarina glauca* Sieber *ex.* Sprengel with an undergrowth of *S. virginicus* and *Chloris gayana* Kunth adjacent to an *A. marina* swamp.

Trapping technique: Battery-operated EVS traps (Rohe and Fall 1979) were used throughout the study and operated without light. Four traps were placed in a straight line, 200 m apart, perpendicular to the prevalent easterly wind direction. A 4 × 4 Latin square design (Cochran and Cox 1957) was used to test the treatments of: 1) no bait, 2) octenol, 3) CO_2, and 4) octenol + CO_2. The traps were rotated so that each treatment occupied each of the 4 linear positions for a single night at both study areas. The traps were run from 1500 to 0900 h.

Baits: CO_2 was released from a 6-kg high-pressure gas cylinder (CIG, St. Leonards, New South Wales, Australia) at 200 ml/min using a pressure regulator (TR74 Carbon Dioxide Regulator; Comweld Group Pty. Ltd., Victoria, Australia) and a fine flow regulator (Nupro B-SS4; Nupro Comp., St. Willoughby, OH). The cyl-

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inder was placed beneath the trap and the gas was led through a 6-mm diam nylon tube to the top of the trap cylinder. Before and after each experiment, the flow rate was measured using a portable flow meter (No. 460 air meter, Dwyer Instruments, Inc., Michigan City, IN).

Octenol was released from microreaction vials (4 ml, Kimble, Division of Owens-Illinois) fitted with plastic lids and neoprene septa. Each lid had a 9-mm diam hole that exposed the underlying septa. A 2-mm hole was bored through each septa and a doubled-over cotton pipe cleaner inserted through the hole such that 8–9 mm extended outside the septum (see "wick out" method of Kline et al. [1991a]). Each vial was filled with octenol then fixed adjacent to the CO_2 tube outlet with tape.

Mosquito collections: Mosquitoes were identified (Marks 1982) and counted. Body size was estimated by wing length (Nasci 1986) from a number ($n \leq 30$) of randomly selected *Ae. vigilax* from each collection. Wing length was measured as the distance from the axilliary incision to the apical margin excluding the fringe scales (Harbach and Knight 1980). In addition, the ovaries of these specimens were removed, and parity determined by examination of their tracheoles (Detinova 1962).

Analysis of data: Catches (transformed to log[n + 1]) and mean wing lengths were analyzed with Statistical Analysis System programs PROC GLM and Means/TUKEY (SAS Institute 1988) for analysis of variance and mean comparisons, respectively. A chi-square test was used to analyze the parity rates by using the Statistical Analysis program PROC FREQ and a t-test (PROC TTEST) was used to analyze mean release rates of octenol (SAS Institute 1988).

RESULTS

Octenol release rates: The mean release rate (±SD) of octenol at Rocky Point was 5.12 ± 0.48 mg/h for octenol alone and 6.60 ± 2.26 mg/h for octenol in combination with CO_2. At the Brisbane airport, the mean ± SD release rate for these treatments was 5.28 ± 0.63 and 6.17 ± 1.06 mg/h, respectively. There were no significant differences between these 2 release rates for both study sites (Rocky Point: $t = -1.28$, $P = 0.29$; Brisbane airport: $t = -1.45$, $P = 0.20$; t-test).

Mosquito collections: At Rocky Point, 2,376 mosquitoes, encompassing 8 species, were collected. *Aedes vigilax* (63.6%) was the most common species. Other species present were *Culex sitiens* Wied. (26.2%), *Aedes funereus* (Theobald) (8.5%), *Cx. annulirostris* (1.4%), *Aedes notoscriptus* (Skuse) (0.4%), *Anopheles annulipes s.l.* Walker (0.1%), *Mansonia uniformis* (Theobald) (0.1%), and *Aedes alternans* (Westwood) (0.05%). At Brisbane airport, 6,916 mosquitoes were captured, consisting of 5 species: *Ae. vigilax* (98.8%), *Cx. sitiens* (1.0%), *Ae. funereus* (0.06%), *Cx. annulirostris* (0.06%), and *Ae. alternans* (0.03%) (Table 1). No male mosquitoes were collected at either site.

Day, site, and treatment effects: Only *Ae. vigilax, Ae. funereus, Cx. annulirostris,* and *Cx. sitiens* were collected in numbers large enough for statistical testing. At Rocky Point, there was no significant difference in catches between days or sites. At Brisbane airport, the only significant difference found was for *Ae. vigilax* between days ($F = 6.64$, $P = 0.02$). Significant treatment differences ($P < 0.05$) were found for all tested mosquitoes at Rocky Point and for *Ae. vigilax* and *Cx. sitiens* at Brisbane airport.

In both study areas, the number of mosquitoes caught in unbaited and octenol-baited traps was very low relative to traps baited with CO_2. For most species, excepting *Ae. vigilax* at Brisbane airport, octenol alone did not significantly increase the collections over the control. Few if any *Culex* were collected by octenol-baited traps.

Using CO_2 alone, 4 of the 8 species were collected in significantly increased numbers relative to unbaited and octenol-baited traps. At Brisbane airport, CO_2 only collected substantial numbers of *Ae. vigilax*. The numbers of *Ae. funereus* and *Cx. annulirostris* were not significantly increased, although *Cx. sitiens* was at both sites (Table 1).

The largest collections of *Ae. vigilax* and *Ae. funereus* were made using CO_2 + octenol-baited traps (Table 1). However, the increase relative to CO_2 was not significant (excepting *Ae. vigilax* at Rocky Point) owing to the high variability of the data. Collections of both *Culex* species decreased with the addition of octenol.

Wing length and ovarian tracheation: Small collections limited statistical analysis in both trials (Tables 2 and 3). There were no consistent or significant trends in wing length or in the proportion with extended tracheoles, thus indicating that the different trapping methods did not selectively collect any particular age grade.

DISCUSSION

From previous studies it has been demonstrated that both CO_2 and octenol (Takken and Kline 1989; Kline et al. 1990a, 1990b, 1991a, 1991b) can serve as an attractant for several mosquito species. The data in this paper demonstrate that CO_2 alone is an attractant for *Ae. vigilax, Ae. funereus, Cx. sitiens,* and *Cx. annulirostris.* The results also show that octenol combined with CO_2

Table 1. Mean catch ± SD per trap per day[1] for different treatments of odor-baited EVS traps at Rocky Point (February 3–17, 1993) and the Brisbane airport (February 18–March 5, 1993). See text for trapping and bait release rates.

Location	Species	Bait			
		None	Octenol	CO_2	Octenol + CO_2
Rocky Point	*Aedes funereus*	4.3 ± 5.1 AB	1.0 ± 1.4 A	8.8 ± 3.0 BC	36.3 ± 19.8 C
	Aedes vigilax	2.0 ± 1.2 A	2.3 ± 1.3 A	85.0 ± 51.6 B	288.3 ± 171.4 C
	Culex annulirostris	0.0 ± 0.0 A	0.0 ± 0.0 A	5.5 ± 2.1 B	3.0 ± 3.6 B
	Culex sitiens	1.3 ± 1.5 A	1.8 ± 1.0 A	95.8 ± 40.3 B	56.8 ± 38.3 B
	Aedes spp.[2]	0.0 ± 0.0	0.0 ± 0.0	4.0 ± 4.3	4.0 ± 4.7
	Anopheles annulipes	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.5	0.5 ± 1.0
	Mansonia uniformis	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.5	0.3 ± 0.5
Brisbane airport	*Ae. funereus*	0.0 ± 0.0 A	0.0 ± 0.0 A	0.5 ± 1.0 A	0.5 ± 1.0 A
	Ae. vigilax	1.8 ± 1.7 A	18.5 ± 7.0 B	654.0 ± 269.4 C	1,034.8 ± 275.1 C
	Cx. annulirostris	0.0 ± 0.0 A	0.0 ± 0.0 A	0.3 ± 0.5 A	0.8 ± 1.0 A
	Cx. sitiens	0.0 ± 0.0 A	0.0 ± 0.0 A	10.3 ± 6.8 B	7.0 ± 8.5 AB
	Aedes spp.[3]	0.0 ± 0.0	0.0 ± 0.0	4.3 ± 4.6	4.5 ± 5.4

[1] $n = 4$ days; means in the same row followed by the same letter are not significantly different ($P > 0.05$); Tukey's multiple range test applied to $\log(n + 1)$-transformed data.
[2] Composed of 3% *Aedes alternans*, 30% *Aedes notoscriptus*, and 67% unidentified damaged *Aedes* spp.
[3] Composed of 6% *Ae. alternans* and 94% unidentified damaged *Aedes* spp.

Table 2. Wing length of female *Aedes vigilax* collected at Rocky Point (February 3–17, 1993) and the Brisbane airport (February 18–March 5, 1993). See text for trapping details.

Location	Bait	*n*	Wing length, mm[1] Mean ± SD	Range
Rocky Point	CO_2	128	2.78 ± 0.243 A	2.26–3.26
	Octenol + CO_2	131	2.84 ± 0.235 B	2.23–3.49
Brisbane airport	Octenol	61	2.69 ± 0.276 A	2.17–3.26
	CO_2	119	2.86 ± 0.256 B	2.14–3.40
	Octenol + CO_2	128	2.81 ± 0.283 B	2.03–3.51

[1] Means followed by the same letter are not significantly different ($P > 0.05$); Tukey's multiple range test.

considerably enhances catches of *Ae. vigilax* and *Ae. funereus*. Octenol by itself is only slightly attractive for *Ae. vigilax*, but seems to have a synergistic effect in combination with CO_2 at Rocky Point. This supports the results of Takken and Kline (1989) and Kline et al. (1990a, 1990b, 1991a, 1991b) with *Ae. taeniorhynchus*. The increase in the numbers of *Ae. funereus* at Rocky Point in the presence of octenol and CO_2 also supports this view; numbers of *Ae. funereus* from Brisbane airport were too small to be conclusive.

The largest numbers of *Cx. sitiens* and *Cx. annulirostris* were collected when CO_2 was used as the sole bait and declined slightly with the addition of octenol. Kline et al. (1990a) proposed that *Culex* species that are ornithophilic do not respond to octenol. Unfortunately, information on the host feeding patterns of *Cx. sitiens* is largely anecdotal (Standfast and Barrow 1968; Kay, unpublished data) and limited (Lee et al. [1989] reported 12 of 15 precipitin tests were positive for mammals). *Culex annulirostris* is well described as a mammalophilic species (Kay et al. 1979), but the small collections of *Cx. annulirostris* in this study are inconclusive.

This is the first study that compares the relationship between the response to CO_2 and octenol with the size and physiological age of mosquitoes. The differences in wing length between treatments, albeit significant, were relatively small and were contradictory for the 2 sites. This suggests that the size of *Ae. vigilax* attracted by CO_2 and by octenol is comparable. Unfortunately, octenol did not increase the proportion of *Ae. vigilax* with extended tracheoles. According to C. Jennings (unpublished data), this fraction does not relate simply to parity, as such extension may occur in newly emerged autogenous females. However, in total both criteria suggest that the same age classes were being collected by all treatments.

Octenol in combination with CO_2 is a better supplement than just CO_2, which is currently used with EVS traps in Australia for the collection of *Ae. vigilax*. This approach would be applicable to sampling low-density populations, for collections for arbovirus assay, or, potentially, for removal trapping of these species. However, collections of *Culex* may be diminished by addition of octenol. Research into the relative attractiveness of different release rates of octenol should be done.

ACKNOWLEDGMENTS

We thank W. Takken and D. L. Kline for their advice given before this study. We are grateful to C. Hohnen of the Queensland Herbarium of the Queensland Department of Environment and Heritage for the identifications of the plant species. We also thank C. Jennings for his assistance in the statistical analysis of the data and G. Hap-

Table 3. Age structure of female *Aedes vigilax* collected at Rocky Point (February 3–17, 1993) and the Brisbane airport (February 18–March 5, 1993) by examination of ovarian tracheation.

Location	Bait	Total	No. dissected	% extended tracheoles[1,2]
Rocky Point	CO_2	340	113	67.3 A
	Octenol + CO_2	1,153	107	67.3 A
Brisbane airport	Octenol	74	62	53.2 A
	CO_2	2,616	112	52.7 A
	Octenol + CO_2	4,139	124	41.9 A

[1] For *Ae. vigilax*, extended tracheoles = parous plus autogenous nulliparous grades.
[2] Percentages with the same letter do not differ significantly ($P > 0.05$) as determined by chi-square test.

good for providing the EVS traps. This study was supported in part by the Local Authorities Research Committee.

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