urnal of the American Mosquito Control Association, 21(4):497–500, 2005

OPERATIONAL NOTE

COMPARISON OF CARBON DIOXIDE- AND OCTENOL-BAITED ENCEPHALITIS VIRUS SURVEILLANCE MOSQUITO TRAPS AT THE SHOAL WATER BAY TRAINING AREA, QUEENSLAND, AUSTRALIA

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ABSTRACT The use of octenol in combination with carbon dioxide (CO_2)-baited encephalitis virus surveillance (EVS) mosquito traps was evaluated under simulated wartime operational conditions during Operation Tandem Thrust (TT01) at the Shoalwater Bay Training area, Queensland, Australia in 2001. A greater number of mosquito species were captured in traps baited with octenol plus CO_2 than those baited with CO_2 or octenol in the saltwater marsh, Freshwater Beach. In the inland environments of Camp Growl and Raspberry Creek, the addition of octenol did not significantly increase the numbers of mosquito species captured. Trap treatment (octenol only, CO_2 only, or octenol plus CO_2) influenced the species captured at Freshwater Beach. More *Ochlerotatus vigilax, Mansonia uniformis,* and *Coquillettidia xanthogaster* were captured in traps baited with octenol plus CO_2, and more *Anopheles* were captured in traps baited with CO_2 only. The most commonly captured (83%) mosquito species in the Shoalwater Bay Training Area during TT01 was the salt marsh breeder and Ross River virus vector, *Oc. vigilax.*

KEY WORDS Octenol, U.S. military, Ross River virus, Australia, *Ochlerotatus vigilax*

Operation Tandem Thrust 2001 was a joint military exercise involving thousands of members of the Australian and United States Armed Forces. The exercise occurred in the 170,000-ha Shoalwa- Bay Training Area (SWBTA) located along the ortheastern coast of Queensland, Australia. The major duty of military medical entomologists deployed on this exercise was the capture and identification of mosquitoes in support of efforts to assess the risk of Ross River virus (RRv), Barmah Forest virus (BFv), and dengue virus transmission among deployed personnel. The threat of virus transmission had previously been established in the SWBTA. During Tandem Thrust 1997 (TT97), there were 9 confirmed clinical cases of RRv and 1 confirmed clinical case of BFv. Additionally, RRv was isolated from 2 of the human cases. Concurrently, RRv was isolated from 4 mosquito species during TT97 (S.E. Cope, unpublished data).

Military entomologists use a wide array of collection and observation techniques to assess the vector-borne disease risk of an area. One tool commonly used is the encephalitis virus surveillance (EVS) mosquito trap baited with carbon dioxide (CO_2). The CO_2-baited EVS trap has been an efficient tool for surveying active mosquitoes that are attracted to CO_2 and light. However, in some environments, the addition of octenol has increased both the numbers and species of mosquitoes captured (Kline et al. 1991, van den Hurk et al. 1997, Rueda et al. 2001). In some cases, this attraction has been so substantial that localized populations of mosquitoes have been reduced simply by setting numerous octenol-baited traps in an area (Kline and Lemire 1998). However, the interaction of octenol with the traditional attractants of light and CO_2 are not completely understood (Kline 1994). Some mosquito species are not attracted to octenol (Beavers et al. 1998). Therefore, when trapping operations occur in an unknown environment, octenol must first be evaluated before any benefit can be attributed to its use.

The purpose of this study was to undertake an evaluation of the use of octenol with EVS traps to determine if it would be useful for the assessment of disease risk during future Tandem Thrust deployments within the SWBTA.

Octenol-baited CO_2 traps were evaluated in three areas of the SWBTA: Raspberry Creek, the northeast section of the Razorback Sector north of the crossing of the East-West road; Camp Growl, the eastern central area of Glen Sector; and Freshwater Beach, the southeast section of the Freshwater Sector (Shoalwater Bay Special Queensland Topographical Map, stock #AUSPEC0155, Australian Defense Topographic Agency, 2001). Raspberry Creek was a densely wooded riparian habitat approximately 20 km from the coast. Camp Growl was a densely wooded habitat 35 km from the coast with no major water sources within a 2 km radius. Freshwater camp was a coastal habitat adjacent to a large saltwater marsh. Three EVS mosquito traps

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Table 1. Mean (SE) of number of mosquito species captured in EVS traps baited with CO_2, octenol, or CO_2 plus octenol in three separate habitats within the SWBTA, Queensland, Australia.

Habitat	Treatment		
	CO_2 + Octenol	CO_2	Octenol
Freshwater Beach	3.67a[1] (0.33)	2.00b (0.00)	1.33b (0.33)
Raspberry Creek	3.00a (1.53)	2.00a (0.58)	1.33a (0.33)
Camp Growl	1.67a (0.33)	0.67ab (0.33)	0.00b (0.00)

[1] Row means followed by the same letter were not significantly different ($P < 0.05$), as determined by Tukey's Studentized Range test.

(BioQip Products Heavy Duty EVS CO_2 Trap Model 2801A, Rancho Dominguez, CA) were placed ≈90 m apart within each habitat for 3 consecutive nights. On each night, 1 trap contained CO_2 (≈0.5 kg-pellets) only, another trap contained only an octenol lure (MA-1000 Octenol Mosquito Attractant Cartridge, Flowtron Inc., Melrose, MA) secured to the fan housing with a rubber band, and a 3rd trap contained both octenol and CO_2. Trap locations were static, but the CO_2 plus octenol treatment combinations were rotated each night so that each trap and location received all treatment combinations 1 time after 3 nights of trapping. Traps were deployed in the evening between 1600 and 1700 h and collected in the morning between 0800 and 0900 h. The number and species of mosquitoes from each trap were recorded daily. All data were analyzed separately by habitat (SAS Institute 1987).

First, the CO_2, octenol, and CO_2 plus octenol trap treatments were compared using a 1-way analysis of variance (ANOVA) on total number of species captured. *Anopheles* spp. were not identified to species because the captured individuals were generally damaged beyond identification. Therefore, in the analysis, *Anopheles* spp. were grouped at the genera level. A Tukey's Studentized Range test was used to compare means. Secondly, a correlation analysis was used to compare numbers of individual species to trap treatments (CO_2, octenol, and CO_2 plus octenol) by habitat. A significant χ^2 ($P < 0.05$) indicated that the data were significantly correlated, meaning that the complex of mosquito species captured was influenced by trap treatment.

Fully 88% of all mosquitoes captured in this study were collected at Freshwater Camp, and 83% of the mosquitoes captured were RRv vector *Ochlerotatus vigilax* (Skuse). Camp Growl was the only inland area where a few *Oc. vigilax* were collected.

Trap treatment influenced the number of species captured within Freshwater Beach ($F = 9.50$, df = 2, 6, $P = 0.014$) and Camp Growl ($F = 19.50$, df = 2, 6, $P = 0.002$), but not within Raspberry Creek ($F = 0.76$, df = 2, 6, $P = 0.508$) (Table 1). At Fresh Water Beach, a higher number of mosquito species was captured in traps baited with a combination of CO_2 plus octenol, but there were no differences in the numbers of species captured in traps baited with only CO_2 or octenol (mean [SD] = 3.67 [0.33], 2.00 [0.00], and 1.33 [0.33], respectively). At Camp Growl, a higher number of mosquito species was captured in CO_2 plus octenol-baited traps when compared with traps baited with only octenol, but traps baited with CO_2 by itself did not significantly capture more mosquitoes than either the CO_2 plus octenol combination or octenol by itself (mean [SE] = 1.67 [0.33], 0.00 [0.00], and 0.67 [0.33], respectively). There were no differences in the numbers of mosquito species captured at Raspberry Creek, regardless of trap treatment for CO_2 plus octenol, CO_2, and octenol (mean [SE] = 3.00 [1.53], 2.00 [0.58], and 1.33 [0.33], respectively).

Trap treatment was correlated with mosquito species captured at Freshwater Beach, but not at Raspberry Creek or Camp Growl (Tables 2–4, respectively). The combination of CO_2 plus octenol at Freshwater Beach captured more *Oc. vigilax*, *Mansonia uniformis* (Theobald), and *Coquillettidia. xanthogaster* (Edwards) than CO_2 or octenol alone. This is in agreement with other studies conducted in southeast Queensland in which it was shown that greater numbers of *Oc. vigilax* and *Aedes funereus*

Table 2. Correlation analysis of EVS traps baited with CO_2, octenol, or CO_2 plus octenol with mosquito species, percent (number captured), at Freshwater Beach, Queensland, Australia.[1]

Species	Treatment		
	CO_2 + Octenol	CO_2	Octenol
Oc. vigilax	61.68 (879)	37.33 (532)	0.98 (14)
Anopheles spp.[2]	29.17 (7)	66.67 (16)	4.17 (1)
Ma. uniformis	100.00 (22)	0.00 (0)	0.00 (0)
Cq. xanthogaster	62.50 (10)	37.50 (6)	0.00 (0)

[1] $\chi^2 = 25.88$, $n = 1{,}487$, df = 6, $P < 0.001$.
[2] *Anopheles* were identified only to genera.

Table 3. Correlation analysis of EVS traps baited with CO_2, octenol, or CO_2 plus octenol with mosquito species, percent (number captured), at Raspberry Creek, Queensland, Australia.[1]

Species	Treatment		
	CO_2 + Octenol	CO_2	Octenol
Ma. uniformis	74.84 (116)	20.00 (31)	5.16 (8)
Oc. kochi	100.00 (7)	0.00 (0)	0.00 (0)
Oc. cunabulanus	60.00 (3)	40.00 (2)	0.00 (0)
Cq. xanthogaster	75.00 (3)	25.00 (1)	0.00 (0)
Oc. procax	100.00 (1)	0.00 (0)	0.00 (0)

[1] $\chi^2 = 4.35$, $n = 172$, df = 8, $P = 0.82$.

(Theobald) were captured when octenol was added to CO_2-baited EVS traps (Kemme et al. 1993). It is important to note that on some trapping nights in Camp Growl only the CO_2 plus octenol combination captured the RRv vector, *Oc. vigilax.*

Traps at Freshwater Beach baited with only CO_2 captured the greatest number of *Anopheles* spp. However, van den Hurk et al. (1997), in far-northern areas of Smithfield, Wyvari Swamp, and Martelli Eubenangee Swamp, Queensland, captured more *Anopheles farauti* (Laveran) in Centers for Disease Control light traps baited with octenol and CO_2 than with CO_2 alone. This situation demonstrates the need to trap with both CO_2 and the combination of CO_2 plus octenol to maximize the numbers and species of mosquitoes captured.

At Camp Growl and Raspberry Creek, the addition of octenol to a CO_2-baited trap did not increase the numbers of species captured or influence the species of mosquitoes captured. However, at these locations, the combination of octenol plus CO_2 did capture numerically higher numbers of mosquitoes. More research is needed at these locations to determine if the addition of octenol does influence the mosquito capture in these areas.

Deployed military medical entomologists, tasked with evaluating the vector-borne disease transmission risk within a given environment, need to have as much information as possible so that informed assessments can be made. However, given the highly mobile and dangerous conditions of military operations, medical entomologists generally do not have adequate time or freedom of movement to thoroughly evaluate each environment encountered in the same way that a long-term research study would. This study, conducted under operational (simulated wartime) conditions, determined that the addition of octenol to CO_2-baited EVS traps did capture the most mosquito species at 1 (Freshwater Beach) of the 3 locations studied in the SWBTA. However, the combination of octenol plus CO_2 did not always capture the highest number of some mosquito species. At Freshwater Beach, more *Oc. vigilax, Ma. uniformis,* and *Cq. xanthogaster* were captured in traps baited with the combination of CO_2 plus octenol than traps baited with only CO_2, but a greater number of *Anopheles* were captured in traps baited with CO_2 only than traps baited with the combination of CO_2 and octenol.

The results presented here provide evidence that octenol in addition to CO_2 will enhance the ability of medical entomologists to assess the disease risk in some areas of the SWBTA. However, given the working conditions inherent to military operations that limit trapping sites and times, plus species specificity to trap treatment, it is recommended that on each trapping night, some traps be baited with CO_2 and others with the combination of CO_2 plus octenol to maximize species capture.

We thank Dr. Allen Miller for critical review of this manuscript.

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Table 4. Correlation analysis of EVS traps baited with CO_2, octenol, or CO_2 plus octenol with mosquito species, percent (number captured), at Camp Growl, Queensland, Australia.[1]

Species	Treatment		
	CO_2 + Octenol	CO_2	Octenol
Oc. vigilax	52.17 (12)	47.83 (11)	0.00 (0)
Oc. procax	100.00 (1)	0.00 (0)	0.00 (0)
Ma. uniformis	100.00 (1)	0.00 (0)	0.00 (0)

$\chi^2 = 1.7$, $n = 25$, df = 2, $P = 0.42$.

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Picture (Left) is a sample of the of the EVS Trap as used in the Preceding Report

(Bucket at top is for Dry Ice)

The EVS (Encephalitis Vector Survey) trap, using carbon dioxide as the primary attractant, was introduced in 1979. It has been subject to evolutionary improvements in the intervening years, and this model incorporates those changes.
The top section is a one-gallon plastic, non-rusting, dry ice container. The wall and lid are insulated with high-density Plastazote foam to prevent overly rapid sublimation of dry ice during operation, and holes in the bottom allow gas to escape. A carrying handle is provided, along with metal chain to facilitate hanging from a tree branch or other object.

Note** these style of traps are predominantly used by entimologists to distinguish the type of vectors existing in the areas they are deployed in.