CARBON DIOXIDE AND 1-OCTEN-3-OL AS MOSQUITO ATTRACTANTS[1]

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ABSTRACT. Interval suction traps were used to study the attractant effect of CO_2 and 1-octen-3-ol on trap catches of mosquito populations at 2 different locations in Florida. There was no significant increase in the numbers of mosquitoes caught when the concentration of CO_2 was increased from 200 to 1,000 cc/min. One-octen-3-ol used by itself attracted mosquitoes in numbers similar to CO_2 released at 200 cc/min. One-octen-3-ol and CO_2 acted synergistically in attracting significantly greater numbers of *Aedes taeniorhynchus, Anopheles* spp. and *Wyeomyia mitchellii* than either bait used singly, although the response of *Culex* spp. to this bait combination was less pronounced. Ceratopogonidae (*Culicoides furens*) and Tabanidae (*Diachlorus ferrugatus, Tabanus nigrovittatus* and *Chrysops* spp.) were also attracted to the combined bait.

INTRODUCTION

Among bloodsucking insects, several different hosts or components of host-odors have been shown to elicit positive anemotactic responses, usually expressed as upwind flight movements (Gillies and Wilkes 1969, Snow 1976, Edman 1979, Vale 1983). Carbon dioxide was found to play an important role in activating (Mayer and James 1969) and, possibly, orientating mosquitoes (Gillies 1980) and biting midges (Nelson 1965) to their hosts.

Recently, field studies on the attraction of tsetse flies to components isolated from ox breath have shown that the use of 1-octen-3-ol (hereafter referred to as octenol) increases the numbers of flies near the odor release point (Vale and Hall 1985a, 1985b). In this paper we present the results of a study to investigate the response of mosquitoes to CO_2 and octenol under field conditions in Florida.

MATERIAL AND METHODS

Study area: Two study areas were used. The first area, Snake Bight, is located at the southern tip of the Everglades National Park, Florida (25°15′N, 81°00′W). Trap sites were located along a hiking trail which ran through mangroves (*Rhizophora mangle* Linn. and *Avicennia nitida* Jacq.) Forty-three species of mosquitoes have been found in the park, but *Aedes taeniorhynchus* (Wiedemann) is by far the most abundant. The species breeds in high numbers throughout the year, subsiding only during the winter months when the relatively low temperatures and lack of rainfall create unfavorable conditions. The second area is in the Lower Suwannee National Wildlife Refuge (29°18′N, 83°3′W) near the Gulf of Mexico. Trap sites were located along a dirt road between a hardwood hammock and a tidal swamp. Thirty-two species of mosquitoes have been recorded from this area (D. L. Kline, unpublished data). During the summer, *Ae. taeniorhynchus, Culex nigripalpus* Theobald and *Psorophora columbiae* (Dyar and Knab) are the most common species, whereas during the winter *Anopheles crucians* Wiedemann and *Culex salinarius* Coq. are dominant in trap catches. In addition, several species of Ceratopogonidae including *Culicoides barbosai* Wirth and Blanton, *C. furens* (Poey) and *C. mississippiensis* Hoffman are seasonally abundant in the tidal swamp (D. L. Kline, unpublished data).

Trapping technique: Battery-operated CDC traps, which had been modified to include an automated interval collection device similar to that described by Standfast (1965), were used throughout the study. The traps were operated without light and with collection intervals set at 2-h periods; the collection containers were changed once daily. Collections were made into 75% isopropyl alcohol. Four traps were placed in a straight line, 200 m apart. Each trap was provided with a different bait daily for a 24-h period according to a Latin square design (4 × 4). The 4 bait combinations were alternated so that each bait occupied each of the 4 linear positions for a single night at each site. The traps were operated for 4 nights at each study area. Although it was realized that wind speed and direction would influence the results, based on Gillies and Wilkes (1972) studies, it was

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determined that the distance between the traps was sufficiently large to eliminate these effects. These investigators showed that mosquitoes responded to CO_2 rates of 250 ml/min and 500 ml/min only up to 20–30 m.

Baits: Carbon dioxide from a 20-lb pressurized cylinder was released near the trap entrance at 200 or 1,000 cc/min through a polyethylene tube. A gas cylinder was placed below each trap and equipped with a pressure gauge and valve and a Gilmont compact flow meter (Gilmont Instruments, Inc., Great Neck, NY). Octenol (Aldrich, Milwaukee, WI) was released from microreaction vials (5 ml, Supelco, Bellefonte, PA) through rubber septa in a manner described by Hall et al. (1984). One vial was fixed to the top of each trap next to the entrance. Under the experimental conditions the release rate was 1.57–2.26 mg of octenol/h. Traps baited with CO_2 released at 200 cc/min were used as standards in both series.

Catches were transformed to log (n+1) for analysis of variance (Sokal and Rohlf 1969). The transformed data were analyzed with Statistical Analysis System (SAS) programs PROG GLM and Means/DUNCAN for analysis of variance and mean comparisons (SAS Institute 1985). Sufficient data for the 2-h intervals were collected for *Ae. taeniorhynchus* and *Wyeomyia mitchellii* (Theobald) at the Everglades so that interval was included as a subplot of day in the statistical analysis for these species.

RESULTS

At Snake Bight, *Ae. taeniorhynchus* outnumbered all other mosquito species. Other species present were *Anopheles atropos* Dyar and Knab, *An. crucians, Wy. mitchellii, Culex* spp. and several species of Ceratopogonidae (*Culicoides furens*) and Tabanidae (*Diachlorus ferrugatus* (Fabr.) and *Tabanus nigrovittatus* Macquart (Table 1). All species were represented entirely by females. At Lower Suwannee several species of Culicidae were represented in the catches, but the numbers were low and variable. Nine species of mosquitoes (*Ae. taeniorhynchus, Ae. infirmatus, Ae. triseriatus, An. crucians, An. quadrimaculatus, Cx. nigripalpus, Cx. pilosus, Psorophora ferox* (von Humboldt) and *Ps. columbiae*) were collected. Only 3 species (Table 1) were collected in numbers large enough for statistical analysis. *Culicoides furens*, however, were fairly abundant. Tabanidae (*D. ferrugatus, T. nigrovittatus* and *Chrysops* spp.) were also collected; they were most abundant in traps baited with both CO_2 and octenol.

CO_2: A 5-fold increase in the output of CO_2 did not result in a significant increase in the total numbers caught, although at Snake Bight the catch of *Ae. taeniorhynchus* occasionally increased more than 2-fold, and also the numbers of *An. atropos* and *An. crucians* increased more than 10-fold.

Octenol: Traps baited only with octenol caught substantial numbers of *Ae. taeniorhynchus, An. crucians, An. quadrimaculatus* Say, *Wy. mitchellii* and Ceratopogonidae. At Snake Bight, the numbers of *Ae. taeniorhynchus* caught in traps baited with octenol were not significantly different from those caught in the standard collections. For most species, however, the numbers of mosquitoes in the octenol-baited traps were less than those caught by the standard. *Culex* spp. were markedly absent from traps baited with octenol only. Ceratopogonidae were attracted in similar numbers to standards and octenol-baited traps, whereas Tabanidae were not attracted to the latter.

CO_2 and octenol: In both study areas, the greatest numbers of mosquitoes were found in traps baited with both CO_2 at 200 cc/min and octenol. At Snake Bight the numbers of *Ae. taeniorhynchus, An. atropos, An. crucians*, Ceratopogonidae and Tabanidae were significantly greater with the combined bait than with the standard. Also, the collections of *Culex* spp. and *Wy. mitchellii* were increased. In Lower Suwannee, the numbers of *An. crucians, An. quadrimaculatus* and Ceratopogonidae were significantly increased, whereas catches of *Aedes* spp. and Tabanidae were greater than those of the standard. In most cases the increase was greater than 2-fold, suggesting a synergistic effect of CO_2 and octenol that caused a dramatic increase in the numbers of certain species of mosquitoes attracted to the traps.

Diel mosquito activity: Daily activity of mosquitoes in relation to bait composition was compared for the 2 most abundant species, i.e., *Ae. taeniorhynchus* and *Wy. mitchellii* (Table 1). Figures 1 and 2 show the activity of females of *Ae. taeniorhynchus* and *Wy. mitchellii* over 2-h intervals during a 24-h period. In October, *Ae. taeniorhynchus* was most active between 1800 and 0800 h, EST, with a clear peak in the early evening. In contrast, *Wy. mitchellii* was most active between 0800 and 2000 h, with a peak between 1100 and 1600 h. Only a few *Wy. mitchellii* were caught between 2200 and 0600 h. In general, all treatment combinations were fairly equally sensitive to diel periodicity of host-seeking behavior. There was no apparent difference in daily activity of either species that could be attributed to bait combination, except perhaps that at 200 cc CO_2/min for *Ae. taeniorhynchus* (intervals from 1000 to 0600) and 1,000 cc CO_2/min for *Wy. mitchellii* (intervals from 2000 through 0800) were eliciting greater response during low activity periods relative to the other

Table 1. Mean catch ± standard error (raw data) per trap per day[1] for different treatments of odor baited CDC traps, Lower Suwannee National Wildlife Refuge (October 10–14, 1986) and Snake Bight, Everglades National Park, FL (October 19–23, 1986).

Species	Bait			
	200 cc CO_2/min	Octenol	1,000 cc CO_2/min	200 cc CO_2/min + Octenol
		Snake Bight		
Aedes taeniorhynchus	5,577.0 ± 1,842.9 a	5,002.0 ± 2,787.2 a	7,382.0 ± 2,582.9 a	32.878.0 ± 4935.4 b
Anopheles atropos	0.5 ± 0.5 a	0.0 ± 0.0 a	8.0 ± 6.2 a	29.0 ± 10.8 b
Anopheles crucians	0.5 ± 0.5 a	0.0 ± 0.0 a	12.3 ± 11.6 a	16.0 ± 7.6 a
Culex spp.	29.5 ± 23.0 ab	0.0 ± 0.0 a	23.0 ± 11.2 ab	55.3 ± 10.2 b
Wyeomyia mitchellii	173.8 ± 64.9 ab	17.8 ± 7.1 a	128.5 ± 74.4 ab	224.0 ± 32.6 b
Ceratopogonidae spp.[2]	3.8 ± 4.1 a	5.7 ± 2.8 a	6.0 ± 3.0 a	272.2 ± 152.1 b
Tabanidae spp.[3]	16.5 ± 6.4 a	0.5 ± 0.5 a	20.0 ± 6.8 a	43.3 ± 8.2 b
		Lower Suwanee		
Aedes taeniorhynchus	0.3 ± 0.3 a	0.5 ± 0.5 a	0.3 ± 0.3 a	4.8 ± 3.8 a
Anopheles crucians	17.5 ± 14.9 a	5.3 ± 2.9 a	8.8 ± 5.2 a	115.5 ± 48.2 b
Anopheles quadrimaculatus	6.5 ± 2.3 a	3.0 ± 1.9 a	7.5 ± 5.3 a	61.5 ± 27.7 a
Ceratopogonidae spp.[4]	27.3 ± 6.9 a	20.0 ± 8.1 a	81.8 ± 18.9 a	271.0 ± 90.5 b
Tabanidae spp.[5]	4.3 ± 1.7 a	0.0 ± 0.0 a	3.5 ± 2.5 a	4.0 ± 1.8 a

[1] n = 4 days; means in the same row followed by the same letter are not significantly different ($P > 0.05$); Duncan's multiple range test (SAS Institute 1985) applied to log-transformed data.
[2] Composed of 100% *C. furens*.
[3] Composed of ca. 50% *D. ferrugatus* and 50% *T. nigrovittatus*.
[4] Composed of ca. 85% *C. furens* and 15% *C. mississippiensis*.
[5] Composed of ca. 40% *D. ferrugatus*, 40% *T. nigrovittatus* and 20% *Chrysops* spp.

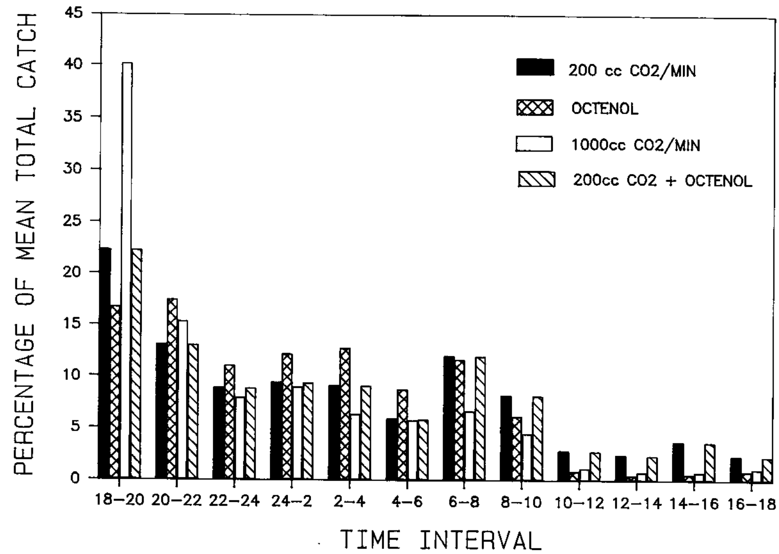

Fig. 1. Daily activity of *Aedes taeniorhynchus* at Snake Bight Trail expressed as the mean catch (%) per 2-h period (n = 4 days).

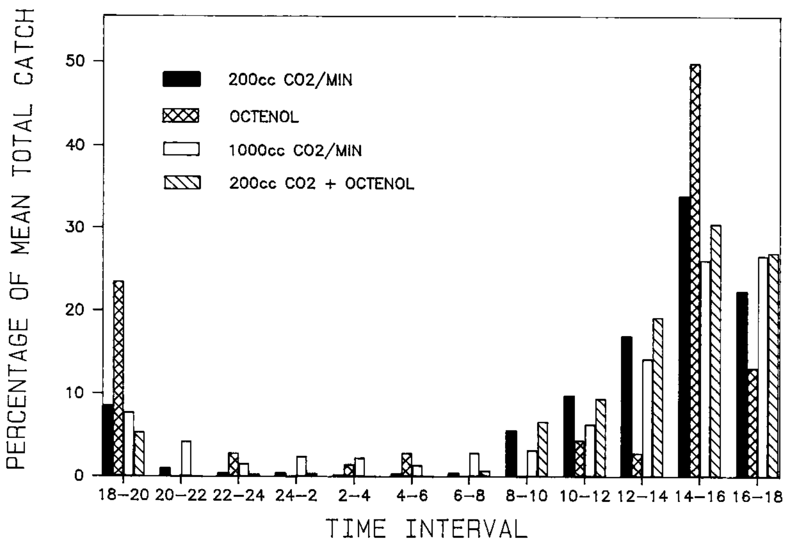

Fig. 2. Daily activity of *Wyeomyia mitchellii* at Snake Bight Trail expressed as the mean catch (%) per 2-h period (n = 4 days).

treatments. Also, 1,000 cc CO_2/min elicited a greater relative response at the peak host-seeking interval (1800–2000) for *Ae. taeniorhynchus*, and the octenol only treatment elicited a greater relative response for *Wy. mitchellii* during 2 intervals (1800–2000 and 1400–1600).

DISCUSSION

The established role of CO_2 as a mosquito attractant (Service 1976) made it feasible to use it as a standard in this study. Gillies (1980), in reviewing the role of CO_2 in host-finding behavior by mosquitoes, concluded that CO_2 elicits 2 distinct actions. First, it acts as an independent attractant; and second, it interacts with warm moist convection currents (emitted by the host) at close range and with other odor factors at a distance from the host. As an independent attractant, the CO_2 emission rate was positively correlated with the range of attraction when studied between 100 and 1,000 cc/min. Stryker and Young (1970) also found an increase in the number of mosquitoes attracted to a point source of CO_2 when releasing it at 400 and 1,250 cc/min, but not above this value. These reported findings are in contrast with those of the present study, possibly because of the high daily variations within each series of this work. Also, at short range from the odor source, the mosquito density may have been low, whereas at a greater distance downwind, the dilution of CO_2 in the odor plume may have resulted in insufficient differences in concentration to affect the mosquitoes there. According to various authors, CO_2 is an activator for several hematophagous insects (Warnes and Finlayson 1985, Gillies 1980, Vale and Hall 1985b). Once activated, the intermittent pulses of CO_2 caused by the natural turbulence of the air within the odor plume serve to guide the insect to the source. In its role as an activator, the concentration of CO_2 would not be important in this process, unlike the concentration of other chemical attractants.

At a distance from the odor source, the attractant effect of CO_2 on mosquitoes can be greatly enhanced by the presence of one or several volatile chemical substances (Gillies 1980). In the present work, we demonstrate for the first time the attractant effect of octenol on mosquito populations in the field. Other than live baits, CO_2 and CO_2 plus human emanations, only L-lactic acid has previously been identified as a mosquito attractant. L-lactic acid attracted mosquitoes in the presence of CO_2, but not alone (Smith et al. 1970). It is suggested, therefore, that L-lactic acid and possibly other compounds act synergistically with CO_2. However, these laboratory results could not be confirmed in the field (Stryker and Young 1970).

The possible role of octenol as an insect attractant was suggested by Buttery and Kamm (1980). Independently from these authors, Hall et al. (1984) demonstrated the role of octenol, identified in the expired breath of oxen, as an attractant for *Glossina*, a blood-feeder. From our studies it appears that at least *Ae. taeniorhynchus* was attracted to octenol as a single bait. However, when combined with CO_2, mosquito responses increased substantially, as did responses in other species of Culicidae. This synergistic effect suggests that responses to odor by mosquitoes might work in a similar way as in tsetse flies (Vale and Hall 1985a). The comparatively low response of *Culex* spp. to octenol might be due to the fact that the majority of these species are ornithophilic, whereas octenol was isolated from the breath of a mammal. More studies are needed to confirm this speculation. The diel activity patterns were similar for all bait combinations tested. The results also demonstrated that effective baits can detect the presence of species which might have gone unnoticed with common CO_2 baited traps, e.g., *Anopheles atropos* and *An. crucians* at Snake Bight.

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