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EVALUATION OF BUTANONE, CARBON DIOXIDE, AND 1-OCTEN-3-OL AS ATTRACTANTS FOR MOSQUITOES ASSOCIATED WITH NORTH CENTRAL FLORIDA BAY AND CYPRESS SWAMPS

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ABSTRACT. Field studies were conducted to determine the responses of mosquitoes found in north central Florida bay and cypress swamps to carbon dioxide (CO_2), light, butanone, and 1-octen-3-ol (octenol), alone and CO_2 in combination with each of the others. The response of these mosquito species to 5 CO_2 release rates (2, 20, 100, 200, and 2,000 ml/min) of CO_2 was also determined. The use of CO_2 resulted in a response in all the species studied; the pattern of response to increasing CO_2 levels varied from species to species. In general, collection size increased as CO_2 release rate increased; however, 5 species (*Aedes dupreei, Anopheles perplexens, Culiseta melanura, Culex erraticus* and *Mansonia titillans*) deviated from this pattern. Collection size of *Ae. dupreei, Cs. melanura,* and *Cx. erraticus* decreased at the 2,000 ml/min release rate. Collection size of *An. perplexens* and *Ma. titillans* remained constant at each CO_2 level to which these species responded. In the CO_2 and light studies, the general pattern for collection size was: CO_2 + light > CO_2 alone > light alone. The combination CO_2 + octenol (2.2 mg/h) resulted in a synergistic response (i.e., greater than the combined response obtained by CO_2 and octenol alone) for all species except *Cs. melanura, Culex nigripalpus,* and *Culex restuans.* Only 2 species (*Aedes atlanticus* and *Aedes canadensis*) responded to octenol in relatively large numbers (i.e., response to octenol alone $\geq 5\%$ of that obtained by using CO_2 alone at the 200 ml/min release rate). Octenol at the release rate tested repelled *Cs. melanura.* The butanone + CO_2 bait combination increased the responses compared to CO_2 alone of *Aedes infirmatus, Culex salinarius, Coquillettidia perturbans,* and *Psorophora ferox,* but decreased the response of *Cs. melanura.*

KEY WORDS Surveillance, baited traps, olfaction, behavior, ecology, mosquitoes, control

INTRODUCTION

In an effort to find alternative control methods to ground and aerial applications of chemical insecticides for mosquito control, several host emanations (carbon dioxide [CO_2], 1-octen-3-ol [octenol], and butanone) have been evaluated against mosquitoes associated with a variety of habitats including salt marshes, mangrove swamps, phosphate mining pits, rice fields, and livestock holding areas. Mosquitoes associated with these habitats varied greatly in their responses to different release rates and combinations of these attractants (Kline 1994).

Responses of mosquitoes associated with bay and cypress swamps to these compounds have not been reported. These mosquitoes are important in the epidemiology of eastern equine encephalitis (EEE) in north central Florida (Choate 1989, Mann 1993). Alternative control methods are needed for these species because during the past 2 decades residential housing developments have increasingly encroached upon these wetland habitats. In many locations where these habitats occur, either no mosquito control program exists, or if one does, current control methods of spraying with chemical insecticides are ineffective due to the physical layout of the housing development. This paper reports the results of our initial efforts to evaluate the potential of incorporating attractants into a control program for these mosquito species.

MATERIALS AND METHODS

Study sites

In 1991, most of the trials were conducted in or around a predominantly red maple (*Acer rubrum* L.) and sweet bay (*Magnolia virginiana* L.) swamp located in Union County, in north Florida (6.4 km east of Lake Butler and 2.3 km north of Florida highway SR100 on Florida highway SR237). This site has had confirmed horse cases of EEE within the past decade (unpublished data). A variety of domesticated animals are kept at this site, including cows, horses, goats, and chickens.

In 1992 and 1993, the studies were conducted at a site located in Alachua County (3.2 km NNW of the intersection of U.S. highway 441 and Florida highway SR121 near the northwestern boundary of Gainesville, FL). Pond cypress (*Taxodium ascendens* Brongniart) is the predominant tree species, but red maple, swamp tupelo (*Nyssa sylvatica* var. *biflora* [Walter] Sargent), and red bay (*Persea borbonia* [L.] Sprengel) are also abundant. No domesticated animals were kept at this site.

Traps

One Model 512, Communicable Disease Center (CDC)-type, 6-V, battery-powered trap (John Hock Co., Gainesville, FL) was suspended from a metal pole ca. 1.5 m above ground level at each trap site. Each trap was equipped with an attractant test com-

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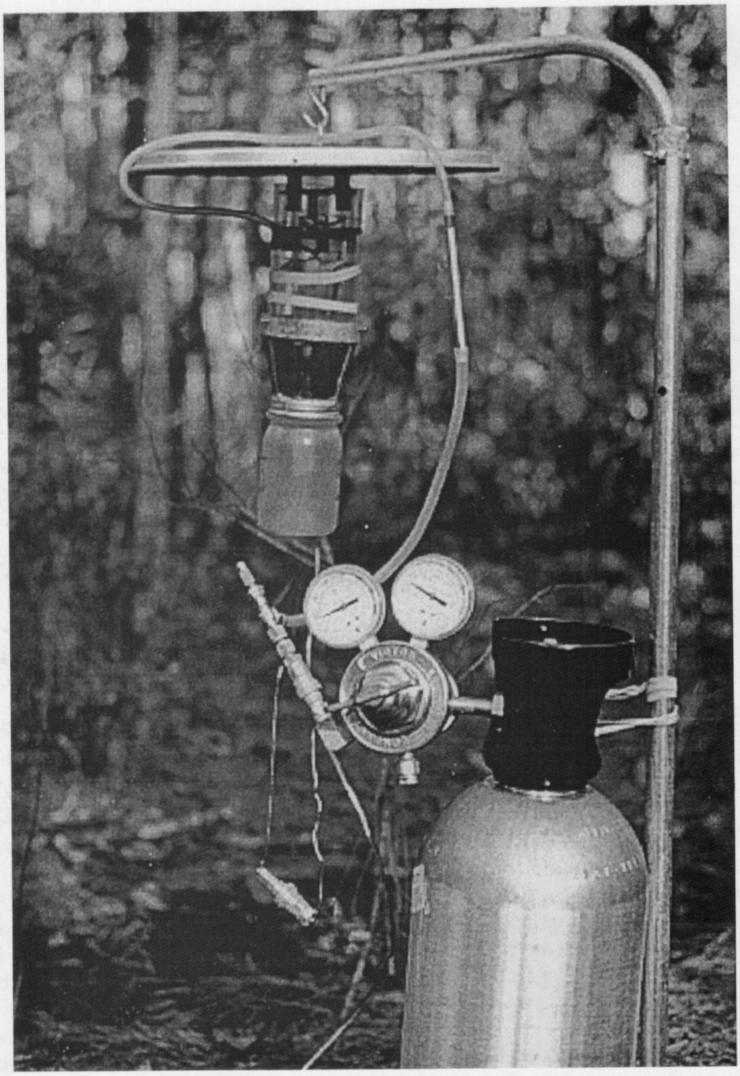

Fig. 1. General configuration of the battery-powered Model 512 Communicable Disease Center (CDC)-type trap with CO_2 tank and metering equipment.

bination. Mosquitoes near the intake were pulled into the trap and blown through a screen funnel into a 568-ml mason jar containing a small piece of resin strip (Industrial Strip™, Bio-Strip, Inc., Reno, NV) impregnated with dichlorvos as the killing agent. All collections were removed daily, taken to the laboratory, sorted, and identified to species.

Attractants

The attractants evaluated in these field trials consisted of light, CO_2, butanone, and octenol. The CO_2 was metered from a 9-kg compressed gas cylinder using a Victor (Model VTS 453B) double-stage regulator (Victoria Equipment Company, Denton, TX) and micrometering valve (Series M, Nupro, Willoughby, OH). Gas flow was monitored using a compact #12 flow meter (Gilmont Instruments, Barrington, IL) and was delivered to its release point, ca. 5 cm from the top trap entrance, via polyethylene tubing. This metering system provided an extremely accurate delivery system that did not fluctuate even under drastic changes in ambient temperature (Fig. 1).

Octenol (Aldrich, Milwaukee, WI) was supplied from a 5-ml microreaction vial (Supelco, Bellefonte, PA) fitted with a plastic lid and neoprene septum using a wick (Dills® 15-cm pipe cleaner) system (Fig. 2). A low-level release rate (2–5 mg/h) was achieved by fashioning the wick so that its length (doubled over) was a few millimeters longer than the distance from the bottom of the vial to the sep-

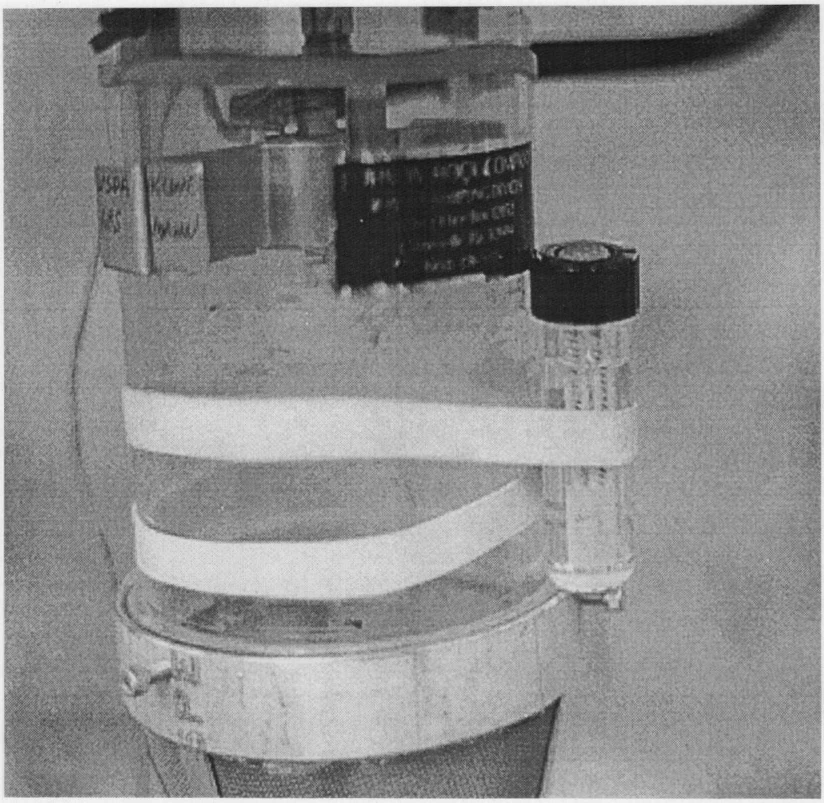

Fig. 2. Illustration of octenol release method and placement of microreaction vial containing octenol near Model 512 Communicable Disease Center (CDC)-type trap entrance.

tum positioned within the lid. This configuration allowed the wick to remain in contact with the permeable septum, releasing the octenol.

Butanone (Aldrich, Milwaukee, WI) was released (ca. 781 mg/h) by a wick extended 2 cm through the lid of a 125-ml amber bottle (Fig. 3). Octenol and butanone containers were secured to the trap near the air intake by means of heavy rubber bands.

Field trials

Carbon dioxide and light studies: Responses to Model 512 traps baited with CO_2 (200 ml/min without light), with light alone, and with CO_2 (200 ml/min) and light were compared in a 3 × 3 Latin square. Trap period, location, and CO_2 release rate were selected to maximize response of *Culiseta melanura* (Coq.). Two replicates were conducted, 1 each in March and April 1993, at the Alachua County site.

Carbon dioxide release rate studies: Five release rates of CO_2 were compared in a 5 × 5 Latin square design utilizing 5 trap sites, separated from each other by ca. 50 m. No light bulb was used in the traps for these trials. Response to release rate, trap site, and trap night were evaluated. Studies were conducted on 5 consecutive nights to minimize variation due to population change. These studies were conducted August 5–10, 1991; October 1–6, 1991; May 4–9, 1992; and October 12–27, 1992. Gas flow was regulated by microregulator to 2, 20, 100, 200, or 2,000 ml/minute. Gas flow was checked at the beginning and end of each sampling period to verify correct flow rate.

Carbon dioxide, butanone, and octenol studies: The responses of mosquitoes to unlighted Model 512 traps baited with butanone alone, butanone + CO_2, octenol alone, and octenol + CO_2, were compared to traps baited with CO_2 alone. All CO_2 treatments were at the 200 ml/min release rate. The average octenol release rate was calculated to be 2.2 mg/h. The average butanone release rate was 0.97 ml (781 mg)/h. Two replicates of a 5 × 5 Latin square design were run in April 1993 at the Alachua County site.

RESULTS

Response to light and CO_2

During 2 3-night trials (18 trap-nights) 6,321 mosquitoes, representing 17 species in 6 genera, were collected (Table 1). The general species response pattern was that collection size for CO_2 +

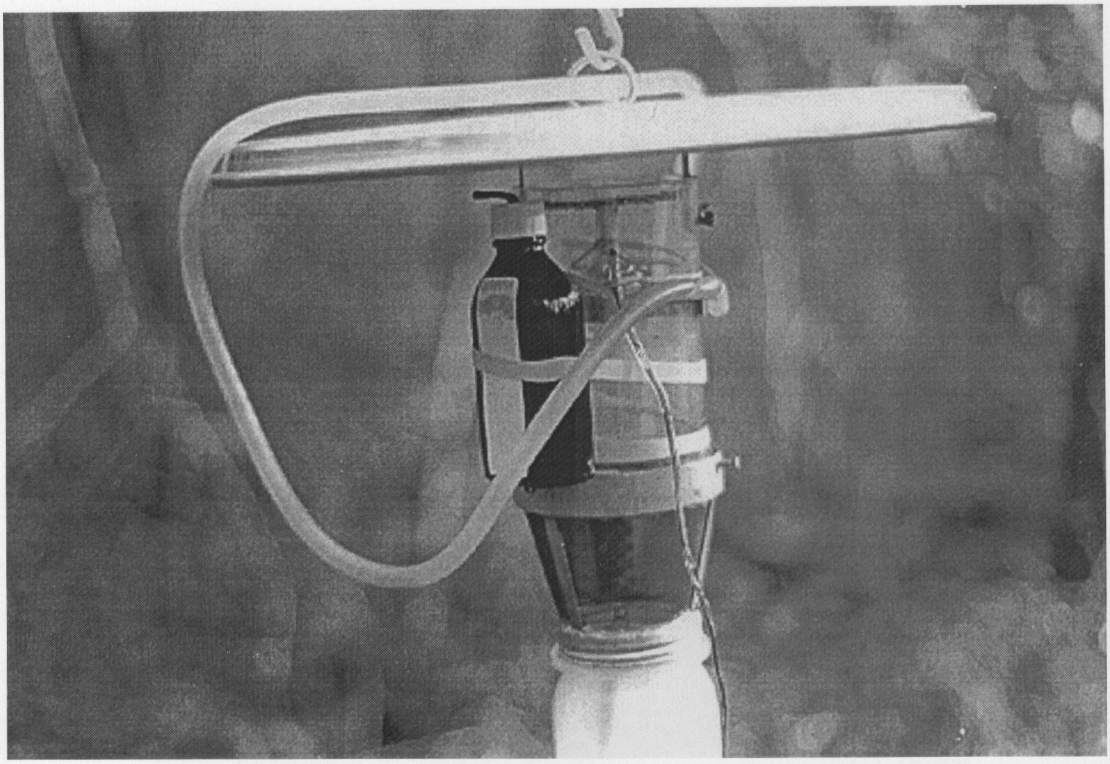

Fig. 3. Illustration of butanone release method and placement near Model 512 Communicable Disease Center (CDC)-type trap entrance.

light > CO_2 alone > light alone. In fact, all 17 species were collected with the CO_2 + light treatment, 12 species responded to CO_2 alone, and only 7 species responded to light alone. Exceptions to this general pattern were that slightly more *Aedes infirmatus* Dyar and Knab and *Culex nigripalpus* Theobald were collected with CO_2 alone than with CO_2 + light; *Ae. infirmatus* was not collected with light alone; more *Culex restuans* Theobald and *Anopheles quadrimaculatus* Say were collected by light alone than with CO_2 alone (no *An. quadrimaculatus* were collected with CO_2 alone).

Table 1. Mean (SE) mosquito responses to Model 512 Communicable Disease Center (CDC)-type traps baited with carbon dioxide (CO_2) (200 ml/min), light, or CO_2 + light (n = 6 trap-nights).

	Treatment		
Species	CO_2	Light	CO_2 + light
Aedes atlanticus	0.50 (0.34)	0.00 (0.00)	0.83 (0.40)
Aedes canadensis canadensis	40.50 (8.38)	3.83 (1.01)	79.83 (23.84)
Aedes infirmatus	2.83 (2.64)	0.00 (0.00)	2.50 (0.85)
Aedes mitchellae	0.00 (0.00)	0.00 (0.00)	0.67 (0.49)
Aedes sticticus	0.33 (0.21)	0.00 (0.00)	1.00 (0.52)
Aedes vexans	0.17 (0.17)	0.00 (0.00)	1.00 (0.68)
Anopheles crucians	318.83 (144.30)	42.83 (12.16)	401.67 (141.15)
Anopheles punctipennis	0.17 (0.17)	0.00 (0.00)	0.50 (0.34)
Anopheles quadrimaculatus	0.00 (0.00)	0.17 (0.17)	0.33 (0.21)
Culex erraticus	0.17 (0.17)	0.00 (0.00)	0.33 (0.33)
Culex nigripalpus	1.00 (0.52)	0.17 (0.17)	0.50 (0.34)
Culex pilosus	0.00 (0.00)	0.00 (0.00)	0.33 (0.33)
Culex restuans	0.33 (0.21)	0.83 (0.65)	3.33 (0.67)
Culex salinarius	24.17 (8.07)	3.33 (0.84)	41.00 (8.45)
Culiseta melanura	21.50 (3.96)	16.67 (5.80)	40.67 (10.74)
Psorophora ferox	0.00 (0.00)	0.00 (0.00)	0.33 (0.21)
Uranotaenia sapphirina	0.00 (0.00)	0.00 (0.00)	0.33 (0.33)

Table 2. Mean (SE) response of the 4 most commonly collected mosquito species in Model 512 Communicable Disease Center (CDC)-type traps baited with carbon dioxide (CO_2), light, or CO_2 + light.[1]

Species	Bait			
	CO_2	CO_2 + light	Light	*P*
Aedes canadensis canadensis	40.50 (8.38)^b	79.83 (23.84)^a	3.83 (1.01)^c	0.0001
Anopheles crucians	318.83 (144.30)^a	401.67 (141.15)^a	42.83 (12.16)^b	0.0001
Culex salinarius	24.17 (8.07)^b	41.00 (8.45)^a	3.33 (0.84)^c	0.0001
Culiseta melanura	21.50 (3.96)^b	40.67 (10.74)^a	16.67 (5.80)^b	0.005

[1] $n = 6$ trap-nights. Means in the same row followed by the same letter are not significantly different; Ryan–Einot–Gabriel–Welsh multiple range test (SAS Institute, Inc., Cary, NC, 1985) applied to ranked data.

Only *Aedes canadensis* (Theobald), *Anopheles crucians* Wied., *Culex salinarius* Coq., and *Cs. melanura* were collected in sufficient numbers for statistical analyses. Results of general linear model (GLM) and means comparisons analyses (means, standard errors, and significant differences at each treatment level) are shown for these species in Table 2. Although all 4 species followed the generalized response pattern, for *Ae. canadensis* and *Cx. salinarius* all treatments were highly significantly different ($P < 0.0001$); for *An. crucians* the CO_2 + light and the CO_2 alone collections were not significantly different from each other, but they were significantly different than the light alone collections ($P < 0.0001$).

For *Cs. melanura,* the CO_2 + light collections were significantly ($P < 0.005$) greater than either the CO_2 alone or light alone collections, which were not significantly different from each other. This unexpected result suggests that surveys whose primary target is *Cs. melanura* could be performed more quickly and cheaply with CDC light traps than with CO_2-baited unlit traps, with little or no decrease in sensitivity, and possibly with an increase in specificity.

Response to various CO_2 release rates

During 4 replicates (100 trap-nights) 13,001 specimens (22 species in 8 genera) were collected. Responses of all species are shown in Table 3. Means, standard errors, and significant differences are shown in Table 4 for the 12 species that were well represented. Both the number of mosquitoes collected and species diversity increased with each increase in CO_2 release rate (Table 3). The general species response pattern was an increase in collection size as the CO_2 release rate was increased. Five

Table 3. Species composition and relative abundance of mosquitoes responding to increasing carbon dioxide (CO_2) release rates.

Species	CO_2 release rate (ml/min)					
	2	20	100	200	2,000	Total
Aedes atlanticus	281	908	1,180	1,935	3,903	8,207
Aedes canadensis canadensis	1	1	3	8	660	673
Aedes dupreei	136	117	299	215	11	778
Aedes fulvus pallens	0	0	0	0	1	1
Aedes infirmatus	3	7	69	98	112	289
Aedes sticticus	0	0	0	0	1	1
Aedes triseriatus	0	0	0	0	9	9
Aedes vexans	0	0	0	0	1	1
Anopheles crucians	4	7	89	101	270	471
Anopheles perplexens	0	0	2	0	2	4
Anopheles punctipennis	0	0	0	0	8	8
Anopheles quadrimaculatus	0	0	0	1	1	2
Coquillettidia perturbans	0	0	7	13	44	64
Culex erraticus	1	2	11	15	8	37
Culex nigripalpus	22	65	101	155	228	571
Culex salinarius	3	17	20	35	60	135
Culiseta melanura	89	375	359	474	228	1,525
Mansonia titillans	0	2	0	2	2	6
Psorophora ciliata	0	0	1	2	5	8
Psorophora columbiae	0	4	9	26	103	142
Psorophora ferox	0	0	7	18	43	68
Uranotaenia sapphirina	0	0	0	0	1	1
Total	540	1,505	2,157	3,098	5,701	13,001

Table 4. Mean (SE) response of the most commonly collected mosquitoes to Model 512 Communicable Disease Center (CDC)-type traps, without light, baited with carbon dioxide (CO_2) at 5 different release rates.[1]

Species	CO_2 release rate (ml/min)					
	2	20	100	200	2,000	$P \leq$
Aedes atlanticus	14.05 (8.93)^{c}	45.40 (41.07)^{c}	59.00 (31.69)^{b}	96.75 (67.99)^{b}	195.15 (123.13)^{a}	0.0001
Aedes canadensis canadensis	0.05 (0.05)^{b}	0.05 (0.05)^{b}	0.15 (0.15)^{b}	0.04 (0.31)^{b}	33.00 (30.65)^{a}	0.005
Aedes dupreii	6.80 (6.75)^{a}	5.85 (5.33)^{a}	14.95 (14.53)^{a}	10.75 (9.52)^{a}	0.33 (0.46)^{a}	0.25
Aedes infirmatus	0.15 (0.11)^{d}	0.35 (0.13)^{c,d}	3.45 (1.47)^{b,c}	4.90 (1.78)^{b}	5.60 (2.07)^{a}	0.0001
Anopheles crucians	0.20 (0.12)^{c}	0.35 (0.17)^{c}	4.45 (2.00)^{b}	5.05 (2.55)^{b}	13.50 (4.22)^{a}	0.0001
Coquillettidia perturbans	0.00 (0.00)^{b}	0.00 (0.00)^{b}	0.35 (0.25)^{b}	0.65 (0.36)^{a,b}	2.20 (1.16)^{a}	0.0001
Culex erraticus	0.05 (0.05)^{b}	0.10 (0.07)^{a,b}	0.55 (0.36)^{a,b}	0.75 (0.34)^{a,b}	0.40 (0.15)^{a}	0.05
Culex nigripalpus	1.10 (0.57)^{c}	3.25 (0.93)^{b}	5.05 (1.51)^{b}	7.75 (2.85)^{b}	11.40 (3.40)^{a}	0.0001
Culex salinarius	0.15 (0.11)^{c}	0.85 (0.39)^{b,c}	1.00 (0.43)^{b}	1.75 (0.73)^{a,b}	3.00 (1.13)^{a}	0.0001
Culiseta melanura	4.45 (1.77)^{b}	18.75 (10.32)^{a,b}	17.95 (6.15)^{a,b}	23.70 (11.52)^{a,b}	11.40 (4.23)^{a}	0.001
Psorophora columbiae	0.00 (0.00)^{b}	0.20 (0.16)^{b}	0.45 (0.20)^{b}	1.30 (0.92)^{b}	5.15 (3.01)^{a}	0.0001
Psorophora ferox	0.00 (0.00)^{b}	0.00 (0.00)^{b}	0.35 (0.26)^{b}	0.90 (0.48)^{b}	2.15 (1.10)^{a}	0.0001

[1] $n = 20$ trap-nights. Means in the same row followed by the same letter are not significantly different; Ryan–Einot–Gabriel–Welsh multiple range test (SAS Institute, Inc., Cary, NC, 1985) applied to ranked data.

species, *Aedes dupreei* (Coquillett), *Anopheles perplexens* Ludlow, *Cs. melanura, Culex erraticus* Dyar and Knab, and *Mansonia titillans* (Walker) deviated from this generalized response pattern. For *Ae. dupreei* the collection size decreased as the CO_2 release rate increased from 2 to 20 ml/min and increased as the CO_2 release rate increased to 100 ml/min; thereafter collection size decreased with each increase in CO_2 release rate with the lowest number of specimens collected at the 2,000 ml/min release rate. Specimens of *An. perplexens* were only trapped at the 100 and 2,000 ml/min release rates (2 specimens at each rate). For *Cx. erraticus,* collection size increased at each step as the CO_2 release rate increased from 2 to 200 ml/min, but decreased at 2,000 ml/min. *Culiseta melanura* followed the same pattern as *Cx. erraticus,* except for a slight decrease in collection size at 100 ml/min. Two specimens of *Ma. titillans* were captured at 3 different release rates (20, 200, and 2,000 ml/min).

Two species (*Culex pilosus* and *Cx. restuans*) were collected in the light + CO_2 series of studies (Table 1), but not in the CO_2 release rate studies. In contrast, 7 species were collected in the CO_2 release rate study, but not in the light + CO_2 study (Table 3). These species were *Ae. dupreei, Aedes fulvus pallens* Ross, *Aedes triseriatus* (Say), *An. perplexens, Coquillettidia perturbans* (Walker), *Psoropha ciliata* (Fabricius), and *Psorophora columbiae* (Dyar and Knab).

Response to octenol, butanone, and CO_2

In the field studies that compared octenol, octenol + CO_2, butanone, and butanone + CO_2 to CO_2 alone in 2 replicates (50 trap-nights), a total of 11,885 mosquitoes (14 species in 6 genera) was collected (Table 5). *Aedes atlanticus* Dyar and Knab, *Ae. canadensis,* and *An. crucians* were clearly most attracted by CO_2 + octenol (Table 6). The response of *Cq. perturbans* to CO_2 + octenol did not differ significantly from this species' response to CO_2 alone or to CO_2 + butanone. The responses of *Ae. infirmatus* and *Cx. salinarius* to CO_2 + octenol did not significantly differ from their responses to CO_2 + butanone. Addition of butanone to CO_2 was effective for decreasing the responses of *Cs. melanura, Ae. atlanticus, Ae. canadensis,* and *An. crucians.* Octenol significantly decreased the response of *Cs. melanura* to CO_2. Octenol alone and butanone alone attracted fewer mosquitoes than any other treatment in all cases. Table 6 presents means, standard errors, and significant differences in response for the most abundant species.

DISCUSSION

Direct comparison of our results with other published studies of mosquito responses to light or CO_2 is difficult because most published studies used different types of adult traps (e.g., New Jersey trap)

Table 5. Mean (SE) response of freshwater swamp mosquitoes to Model 512 Communicable Disease Center (CDC)-type traps (no lights) baited with combinations of butanone, 1-octen-3-ol (octenol), and carbon dioxide (CO_2) (n = 10 trap-nights).

Species	Treatment				
	Butanone	Octenol	CO_2	Butanone + CO_2	Octenol + CO_2
Aedes atlanticus	0.80 (0.33)	5.50 (2.20)	6.20 (2.71)	5.20 (1.84)	37.10 (11.97)
Aedes canadensis	0.60 (0.31)	4.50 (1.42)	55.10 (12.95)	48.60 (10.85)	224.30 (41.42)
Aedes infirmatus	0.00 (0.00)	0.10 (0.10)	0.80 (0.49)	2.20 (0.77)	4.10 (1.67)
Aedes mitchellae	0.00 (0.00)	0.00 (0.00)	0.10 (0.10)	0.00 (0.00)	0.00 (0.00)
Aedes vexans	0.00 (0.00)	0.00 (0.00)	0.10 (0.10)	0.10 (0.10)	0.20 (0.13)
Anopheles crucians	0.60 (0.27)	4.40 (1.86)	106.30 (47.90)	90.70 (31.00)	446.40 (122.32)
Anopheles punctipennis	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.10 (0.10)
Anopheles quadrimaculatus	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.80 (0.49)
Coquillettidia perturbans	0.10 (0.10)	0.30 (0.30)	4.50 (1.67)	8.50 (2.62)	20.50 (11.05)
Culex nigripalpus	0.00 (0.00)	0.00 (0.00)	0.10 (0.10)	0.00 (0.00)	0.00 (0.00)
Culex restuans	0.00 (0.00)	0.00 (0.00)	0.10 (0.10)	0.00 (0.00)	0.00 (0.00)
Culex salinarius	0.00 (0.00)	0.60 (0.40)	16.60 (4.93)	23.10 (13.78)	42.90 (10.05)
Culiseta melanura	1.10 (0.35)	1.00 (0.33)	13.50 (5.28)	8.30 (1.54)	1.20 (0.33)
Psorophora ferox	0.00 (0.00)	0.00 (0.00)	0.10 (0.10)	0.40 (0.31)	0.40 (0.27)

or dry ice as a source of CO_2. However our data agree with the general trends that have been published. For example, our data agree with the general observation that, for most mosquito species, collections are greatly increased (8–30 times) when light traps are baited with CO_2 and compared to unbaited light traps. Huffaker and Back (1943) caught approximately 8 times as many mosquitoes in New Jersey light traps baited with about 3 lb of dry ice than in traps without CO_2, but they noted that the relative order of abundance of the different species was altered by the addition of dry ice. A direct comparison between the attractiveness of light and dry ice showed that dry ice-baited light traps caught about 30 times as many mosquitoes as light only traps (Miller et al. 1969). In Vietnam, the addition of about 4–5 lb of dry ice in a perforated plastic bag increased the catch of mosquitoes in a CDC light trap about 26 times (Herbert et al. 1972). In Malayasia, Parsons et al. (1974) found that the addition of CO_2 to light traps greatly increased the catch of mosquitoes. In trials conducted in Florida, Georgia, and North Carolina (Newhouse et al. 1966), catches from 72 light trap-nights with dry ice were compared with those from 116 light trap-nights without dry ice. In the 3 states the catches of mosquitoes from CO_2-baited light traps were 4, 6, and 6 times, respectively, more than from unbaited light traps. For some species the increase in numbers was obvious. For example, about 20 times for *Cx. salinarius, Wyeomyia mitchellii* (Theobald), *Ae. atlanticus,* and *Aedes tormentor* Dyar and Knab; about 33 times for *Psorophora ferox* (Von Humboldt); and 53 times for *Ae. canadensis.* Other examples that support this general trend are reviewed by Service (1993).

Published reports differ on whether traps baited with a combination of light + CO_2 result in larger mosquito collections than traps baited only with CO_2. In Vietnam, Herbert et al. (1972) found that the mosquito catch was almost doubled when the light was removed and the traps baited only with dry ice. In this Vietnam trial it appeared that light repelled some mosquito species. Stryker and Young (1970), in studies conducted in South Carolina, concluded that little was gained by the addition of light to traps using CO_2. Reisen et al. (1983) caught significantly more female *Culex tarsalis* Coq. in CDC traps baited with 1–2 kg dry ice when the bulb was removed than in traps retaining the bulb. In contrast, Newhouse et al. (1966) found that in addition to increasing the mosquito catch size, dry ice also resulted in a 19–25% increase in the number of species collected. In studies conducted in New York, Magnarelli (1975) showed that dry ice-baited CDC traps caught more mosquitoes than light traps alone, but dry ice added to lighted CDC traps generally gave the largest catches.

An obvious advantage of using cylinders for dispensing CO_2 to traps is that release rates can be regulated, an important consideration if it is suspected that different species are attracted to different emission rates. Uniform release of gas, however, necessitates a sensitive regulatory valve system and meters to control and measure flow rates. Therefore, few studies have been reported in the literature on the responses of mosquitoes to various release rates of CO_2. Even in the reported studies (Carestia and Savage 1967, Carestia and Horner 1968, Graham 1969), difficulties were experienced in controlling flow rates. By using a double-stage regulator, microregulator, and flow meter in our studies, we were able to maintain a CO_2 flow rate as low as 2 ml/min even if ambient temperature changes were drastic. Reeves (1953) used CO_2 release rates of ca. 25, 250, and 2500 ml/min from gas cylinders to simulate the average amounts of CO_2 expired by a chicken, human, and horse or cow, respectively. Adults of *Aedes nigromaculis* Ludlow, a zoophilic species, were attracted in in-

Table 6. Mean (SE) response of the most commonly collected species to Model 512 Communicable Disease Center (CDC)-type traps, without light, baited with 1-octen-3-ol (octenol), octenol + carbon dioxide (CO_2), butanone, butanone + CO_2, or CO_2 alone. All CO_2 release rates are 200 ml/min.[1]

	Bait					
Species	Butanone	CO_2	CO_2 + butanone	CO_2 + octenol	Octenol	$P \leq$
Aedes atlanticus	0.80 (0.33)^b	6.20 (2.71)^b	5.20 (1.84)^b	37.10 (11.97)^a	5.50 (2.20)^b	0.0005
Aedes canadensis canadensis	0.60 (0.31)^b	55.10 (12.95)^b	48.60 (10.85)^b	224.60 (41.42)^a	4.50 (1.42)^b	0.0001
Aedes infirmatus	0.00 (0.00)^b	0.80 (0.49)^b	2.20 (0.77)^{a,b}	4.10 (1.67)^a	0.10 (0.10)^b	0.01
Anopheles crucians	0.60 (0.27)^b	106.30 (47.90)^b	90.70 (31.00)^b	446.40 (122.32)^a	4.40 (1.86)^b	0.0001
Culex salinarius	0.00 (0.00)^b	16.60 (4.93)^b	23.10 (13.78)^{a,b}	42.90 (10.05)^a	0.60 (0.40)^b	0.0005
Culiseta melanura	1.10 (0.35)^b	13.50 (5.28)^a	8.30 (1.54)^{a,b}	1.20 (0.33)^b	1.00 (0.33)^b	0.0005
Coquillettidia perturbans	0.10 (0.10)^b	4.50 (1.67)^{a,b}	8.50 (2.62)^{a,b}	20.50 (11.05)^a	0.30 (0.30)^b	0.05

[1] n = 10 trap-nights. Means in the same row followed by the same letter are not significantly different; Ryan–Einot–Gabriel–Welsh multiple range test (SAS Institute, Inc., Cary, NC, 1985) applied to raw data.

creasing numbers to the higher concentration of gas, whereas more adults of *Culex quinquefasciatus* Say (as *pipiens fatigans* Wied.), an ornithophilic species, were caught with the lowest release rate; *Cx. tarsalis,* which changes its feeding habits during the course of a year, was attracted in large numbers by all 3 release rates, although a slight increase in numbers was associated with increased release rates. Although Carestia and Horner (1968) encountered some difficulties in maintaining a constant flow rate, they attempted to determine the response of mosquitoes to CO_2 flow rates of 25, 50, 125, 250, and 500 ml/min. The numbers caught by these investigators usually increased with greater CO_2 levels, but they concluded that in practice a minimum discharge of about 125 ml/min was necessary to produce a significant increase in catch size. Gas cylinders and controlled release rates of CO_2 were also used by Pfuntner et al. (1988) in comparing catches of *Cx. tarsalis, Culex quinquefasciatus* Say, and *Culex stigmatosoma* Dyar in traps (Pfuntner 1979) placed at different heights (2, 5, and 10 m). Only with *Cx. tarsalis* did the numbers increase with increasing release rates.

Takken and Kline (1989) reported for the 1st time from field experiments that octenol had potential as a mosquito attractant. Later Kline et al. (1990a, 1990b, 1991a, 1991b) conducted a series of field trials that included a variety of habitats and mosquito species with unlit Model 512 traps baited with various combinations of attractants including CO_2, octenol, octenol + CO_2, octenol + butanone + CO_2, lactic acid + CO_2, lactic acid + octenol + CO_2, honey, phenols, and phenols + octenol. Not all attractant combinations were included in each study. Data obtained during these studies showed that different mosquito species sometimes responded differently to these chemicals. The basic response pattern was that very few species were attracted in any numbers to octenol alone, but when octenol and CO_2 were used together, a synergistic effect apparently occurred and a 2-fold or greater catch was obtained with most species of *Aedes, Psorophora, Anopheles, Coquillettidia,* and *Mansonia.* However, *Culex* species showed little attraction to either octenol alone or in combination with CO_2. In contrast to these generalizations, *Aedes taeniorhynchus* (Wied.) and *Cq. perturbans* seemed to respond to octenol alone. The presence of butanone seemed to decrease collections of all species.

These general trends have also been observed in field studies conducted near Queensland, Australia (Kemme et al. 1993). Four estuarine-associated species (*Aedes vigilax* (Skuse), *Aedes funereus* (Theobald), *Culex annulirostris* Skuse, and *Culex sitiens* Wied.) were collected in numbers large enough for statistical analysis. These investigators concluded octenol alone (5 mg/h) was only slightly attractive for *Aedes* spp. Few *Culex* were collected by the octenol-baited traps. Using CO_2-baited (200 ml/min) traps, all 4 species were collected in significantly increased ($P < 0.05$) numbers. The largest collections of *Ae. vigilax* and *Ae. funereus* were made using CO_2 + octenol-baited traps. The increase relative to CO_2 alone was statistically significant only for *Ae. vigilax* because of the high variability of the data. Collections of both *Culex* species decreased with the addition of octenol to CO_2-baited traps.

The obvious conclusion that can be made from our data and other published studies is that different species respond differently to these attractants. Knowledge of the response pattern of each species is important if we are to develop a successful strategy to control targeted species with baited traps or targets. In the case of these swamp-associated species, a strategy to control *Ae. canadensis* would be quite different than one with *Cs. melanura* as the target species. Although unlighted traps baited with CO_2 + octenol would be effective for *Ae. candensis,* a trap baited with CO_2 + light would be more effective for *Cs. melanura.* Many more studies are needed on the responses of these species to different release rates of the attractants reported in this study and candidate attractants that we are developing before a successful strategy can be developed for any species.

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