EFFECTIVENESS OF CARBON DIOXIDE AND L(+) LACTIC ACID IN MOSQUITO LIGHT TRAPS WITH AND WITHOUT LIGHT[1, 2]

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Introduction. In an attempt to develop more effective methods for surveillance of medically important species of the family Culicidae (Diptera), variations in trap design have received great attention in recent years (Bidlingmayer, 1967; Defoliart and Morris, 1967; Gillies and Snow, 1967; Sommerman and Simmet, 1967; and Vickery *et al.*, 1966). Investigations have revealed increased activity and feeding response of mosquitoes when they are exposed to carbon dioxide gas, warmth, or various skin emanations, primarily the various esters of lactic acid (Acree *et al.*, 1968; Carestia and Savage, 1967; Grant, 1969; Khan *et al.*, 1967; and Thompson and Brown, 1955). The L(+) isomer of lactic acid in minute amounts has been shown to be a strong attractant to female *Aedes aegypti*.[5]

This study was an attempt to ascertain the effectiveness of carbon dioxide and L(+) lactic acid as attractants when used in various levels and combinations under field conditions.

Materials and Methods. A salt marsh formed by Goose Creek, located along the eastern boundary of Charleston Army Depot, Charleston, South Carolina, was surveyed from 8 September through 12 October 1969. Three standard New Jersey light traps (FSN 3740-607-0337) with 25-watt bulbs and four battery-operated miniature light traps (FSN 6545-089-3766) were positioned along the marsh boundary. The latter traps are similar in design to the CDC miniature trap, but operate on a 6-volt wet-cell system, utilizing a 6-volt light bulb rather than a 4-volt bulb.

A standard New Jersey trap, also located along the marsh boundary, was used as the control trap. The remaining traps were altered each night according to a planned schedule to conform to a specific gas-acid-light (GAL) treatment. Three levels of gas, five levels of L(+) lactic acid, and the presence or absence of light were tested, totaling 30 treatment combinations (Table 1). A schedule was designed to rotate the treatments along the trap sequence each night.

The mosquito population was considered homogeneous because of the similar salt marsh habitat along the area boundary.

Commercial grade (99.5 percent) carbon dioxide gas was released at three rates with Matheson 1L-AF, 1L-BF, and 1L-CF regulator-flowmeters; 400, 1,250, and 2,470 cc/min. respectively. Matheson size 3 cylinders containing 8 pounds of carbon dioxide at 1,500 psig were used in all tests. Gross weight of a full cylinder was approximately 38 pounds. The flowmeters were calibrated by volumetric displacement at the lowest rate and with a wet test gas meter at the higher rates.

[1] Mention of a proprietary product does not imply endorsement by the U. S. Army. The opinions contained herein are those of the authors and should not be construed as official or reflecting the views of the Department of the Army.

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TABLE 1.—Treatment combinations of carbon dioxide, L(+) lactic acid, and light.

CO_2 Flow Rate, cc/min.					
	0.0	0.001	0.005	0.01	0.1
2470	L+ L—	L+ L—	L+ L—	L+ L—	L+ L—
1250	L+ L—	L+ L—	L+ L—	L+ L—	L+ L—
400	L+ L—	L+ L—	L+ L—	L+ L—	L+ L—
L+=Light on					
L—=light off					

The five levels of L(+) lactic acid tested were 0.00, 0.001, 0.005, 0.01, and 0.1 ml. Measured quantities of L(+) lactic acid dissolved in acetone were placed in two-necked, 300 ml flasks and swirled about the inner surface. The acetone was allowed to evaporate. Rubber tubing directed the gas flow from the flowmeter to one neck of the flask, and a second tubing routed the gas from the second neck to the trap motor housing below the fan intake. This prevented the gas mixture from being drawn directly into the trap, yet released the mixture near the intake portion of the trap. On those tests where L(+) lactic acid was not used, the flasks were by-passed.

Each day the flasks were washed in soapy water and rinsed in acetone. Trap collections were made overnight between 1900 and 0700 hours. Each morning the kill jars were collected and the mosquitoes were counted and packaged for shipment to the Third US Army Medical Laboratory for species identification.

RESULTS AND DISCUSSION. No problems materialized concerning the relative effectiveness of the New Jersey traps and the battery-operated traps. Night catches of the two types of traps were comparable. Occasional irregularities did occur due to wind and scattered rain showers; however, identical treatments to all traps and pooling of the data nullified minor differences.

Carestia and Savage (1967) reported considerable difficulty in maintaining an even gas flow with the 1L series of regulators. During preliminary testing we encountered some difficulty, but thereafter the regulators operated satisfactorily. The carbon dioxide cylinders were satisfactory in size and handling characteristics.

Two approaches were used in the statistical analysis of the data. The first approach was direct, with no adjustments for differences between days; this avoided the problem of possible over or under-adjustment of treatment values. Separate analyses of variance were performed for the mosquitoes caught. Twenty analyses of variance were selected for those days in which treatment replicates occurred. The error terms were pooled and Duncan's multiple range and Multiple F tests were performed (Duncan, 1955).

Significance at the .01 level occurred between those treatments using some combination of L(+) lactic acid, gas, and light in comparison with light alone (control) in 14 of 20 cases.

In five of the six remaining cases, significance at the .01 level was observed as follows:

Gas, cc/min.	Light	Acid, ml
1250	+	—
1250	+	—
2470	+	—
2470	+	0.1
over 1250	+	0.005
over 2470	+	0.005
over 1250	—	—
over 400	+	0.1

No significance at the .01 or .05 level was observed in the remaining case.

The second approach supported the above data. Each day treatments within a day were compared against the control trap by fitting a straight line forced through the origin of the plot of each treatment versus its corresponding control. Table 2 shows the b-values of the plotted slopes. The slope lines were then extended to a theoretical control trap catch of 160 mosquitoes. The corresponding theoretical captures are plotted in Fig. 1.

From inspection, the capture values drop off as the flow rate of carbon dioxide is decreased. The earlier mentioned trend of increased L(+) lactic acid decreasing the mosquito catch may be observed in Figure 1.

Table 3 lists the different species collected at various flow rates of carbon dioxide, disregarding the presence or absence of L(+) lactic acid. Twenty-eight species of mosquitoes were collected. Thirteen species of mosquitoes were common to the test traps and the control trap. An additional 13 species were collected only in the presence of carbon dioxide. *Culiseta inornata* and *Uranotaenia sapphirina* were collected in the standard New Jersey control trap.

Sixty-seven light trap collections performed by the Charleston Army Depot Post Engineers from March through November of 1969, resulted in a total of 28 species of mosquitoes and 6,421 specimens. *Psorophora howardii* was not collected by their surveillance program. In contrast, over 13,000 mosquitoes were collected in one night during this study, doubling the standard surveillance program in total number in a single night.

An examination of the response of various species to carbon dioxide gas at three flow rates indicates difference between the 400 cc/min. rate and 1250 cc/min., increasing the gas flow to 2470 cc/min. did not increase the capture ratio to warrant the greater release rate.

TABLE 2.—b-Values of Slope regression plots.

Gas Flow, cc/min.		L(+) Lactic Acid Level (ml)				
		0.00	0.001	0.005	0.01	0.1
Light On	2470	43.25	28.34	*	13.26	26.75
	1250	24.50	17.49	2.82	16.64	4.90
	450	16.32	16.75	3.23	6.24	6.17
Light Off	2470	16.89	7.49	*	13.25	4.14
	1250	12.50	7.16	31.39	*	13.91
	450	10.82	3.92	11.50	1.05	14.33

* Deleted; data insufficient.

Table 4 is a list of the ten most numerous species (based on collection numbers) illustrating response to treatment type. Dosage levels of gas and acid were pooled within each column. Direct comparison of gas to gas-acid treatment did not reveal any marked preference among the species for either series of treatments. Three species of mosquitoes were collected in greater numbers in the gas-acid-light treatment than in the gas-light. Only one species, *Aedes vexans,* was captured in greater numbers in both gas-acid and gas-acid-light treatments, indicating a positive response for L(+) lactic acid. Similarly *Mansonia perturbans* was collected only from traps emitting carbon dioxide gas. *Culex quinquefasciatus* was caught in greater numbers in the absence of light. Miller *et al.* (1969) working in Thailand reported similar results.

CONCLUSION. The addition of light to a mosquito trap utilizing carbon dioxide gas is not a necessary component in achieving a representative sample of the mosquito population. The addition of L(+) lactic acid to the carbon dioxide gave no discernible beneficial effect, except with *A. vexans.* The number of species of mosquitoes was doubled in traps utilizing

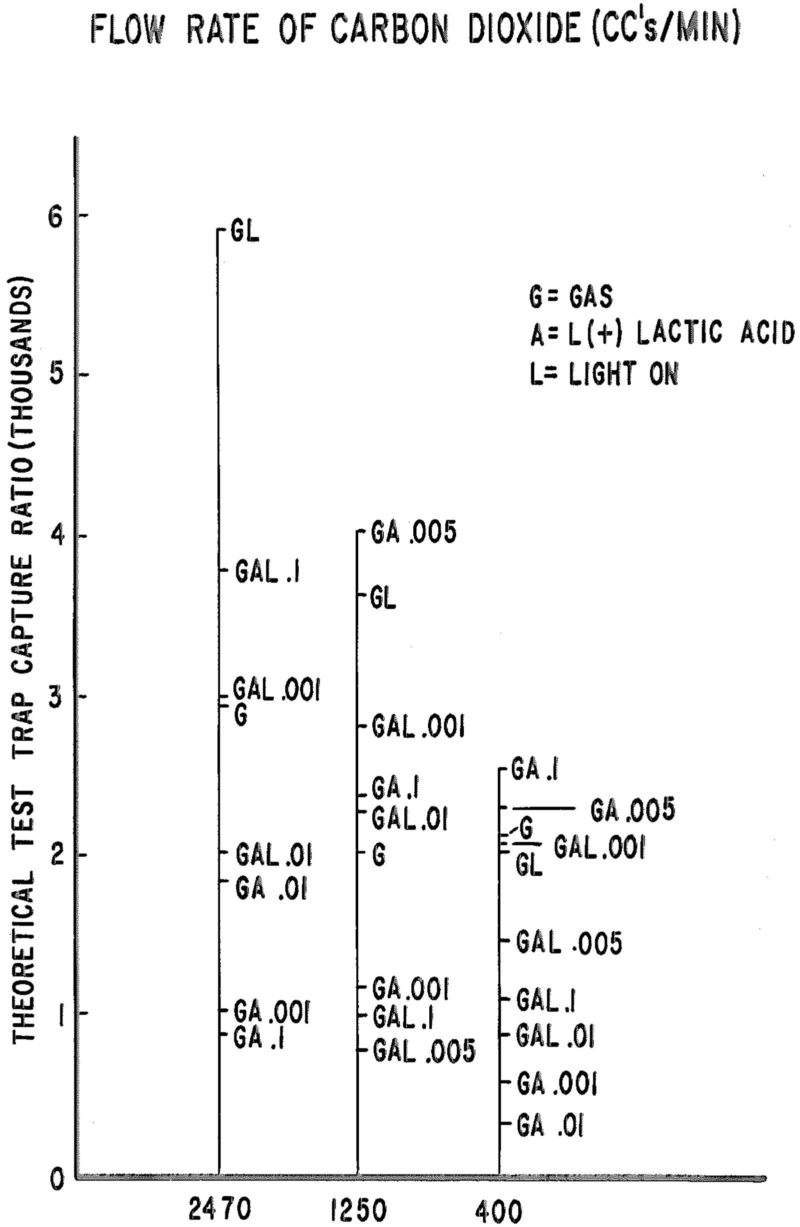

FIG. 1.—Theoretical mosquito capture per trap night for GAL treatment combination compared with the theoretical control trap capture of 160 mosquitoes per trap night.

TABLE 3.—Average number of mosquitoes caught per trap night at each carbon dioxide gas rate disregarding presence or absence of L(+) lactic acid, Charleston Army Depot, Sept.–Oct. 1969.

Species	Gas Flow, cc/min. 2470	1250	400	Total	Control
Aedes					
atlanticus-tormentor	0.00	0.00	0.03	0.01	0.00
canadensis	0.00	0.03	0.00	0.01	0.00
dupreei	0.00	0.00	0.03	0.01	0.00
infirmatus	0.00	0.03	0.00	0.01	0.00
mitchellae	0.06	0.12	0.03	0.07	0.00
sollicitans	123.50	103.80	69.70	99.30	8.76
sticticus	0.00	0.00	0.06	0.02	0.00
taeniorhynchus	145.20	127.60	82.60	118.90	6.24
vexans	5.79	6.91	4.22	5.67	0.41
species*	0.36	0.35	0.09	0.27	0.88
Anopheles					
crucians	12.20	10.70	6.38	9.80	0.76
punctipennis	0.52	0.56	0.06	0.38	0.00
quadrimaculatus	0.24	0.21	0.47	0.30	0.06
species	0.00	0.00	0.19	0.06	0.06
Culex					
nigripalpus	2.33	4.29	2.78	3.15	0.24
quinquefasciatus	1.33	1.50	0.78	1.21	0.24
restuans	8.12	4.29	1.66	4.72	1.35
salinarius	448.80	463.30	323.40	413.30	22.0
tarsalis	0.03	0.00	0.00	0.01	0.12
territans	0.18	0.03	0.00	0.07	0.12
(*Melanoconion*) sp.	0.30	0.15	0.00	0.15	0.00
species	0.03	0.41	0.47	0.30	1.41
Culiseta					
inornata	0.00	0.00	0.00	0.00	0.06
melanura	0.03	0.00	0.00	0.01	0.18
Mansonia					
perturbans	0.42	1.32	1.53	1.09	0.00
Psorophora					
ciliata	0.61	0.35	0.22	0.39	0.00
confinnis	235.80	207.80	179.80	208.10	16.50
cyanescens	0.03	0.00	0.00	0.02	0.00
ferox	0.03	0.00	0.00	0.01	0.00
howardii	0.00	0.06	0.00	0.02	0.00
species	0.03	0.03	0.13	0.06	1.00
Uranotaenia					
sapphirina	0.00	0.00	0.00	0.00	0.53
Number of trap nights	33	34	31	98	17

* Males or unidentifiable specimens.

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TABLE 4.—Average number of the ten most numerous species collected per trap night at Charleston Army Depot, Sept.-Oct. 1969. Treatment levels of carbon dioxide and L(+) lactic acid were combined.

Species	Type Treatment[1]					
	G	GA	GL	GAL	Total	Control
Aedes						
sollicitans	81.70	72.40	183.60	99.40	99.30	8.76
taeniorhynchus	64.00	92.60	178.10	143.70	118.90	6.18
vexans	1.53	3.26	6.57	9.31	5.67	0.41
Anopheles						
crucians	4.93	5.97	15.00	13.40	9.80	0.76
Culex						
nigripalpus	3.53	2.38	5.57	2.78	3.15	0.24
quinquefasciatus	2.53	1.47	0.36	0.75	1.21	0.24
restuans	7.80	2.85	2.71	5.97	4.71	1.35
salinarius	337.30	369.30	573.00	424.20	413.30	22.00
Mansonia						
perturbans	0.07	1.82	1.50	0.67	1.09	0.00
Psorophora						
confinnis	209.10	137.00	372.70	210.70	208.10	16.50
Number of trap nights	15	33	14	36	98	17

[1] G=Carbon dioxide.
GA=Carbon dioxide—L(+) lactic acid.
GAL=Carbon dioxide—L(+) lactic acid—light.
Control=Standard New Jersey light trap.

carbon dioxide gas as compared to light alone.

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