

# EFFECTS OF RELEASE RATES ON THE RANGE OF ATTRACTION OF CARBON DIOXIDE TO SOME SOUTHWESTERN ONTARIO MOSQUITO SPECIES

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**ABSTRACT.** The effects of release rates of 0, 250, 500, 1,000 and 4,000 ml/min on the range of attraction of carbon dioxide to some southwestern Ontario mosquito species was determined using ramp traps placed at 3, 7, 11, 15 and 19 m from a central pressurized cylinder. For female *Aedes vexans*, spring *Aedes* spp. and *Anopheles walkeri*, an increase in the release rate of CO<sub>2</sub> from 1,000 to 4,000 ml/min resulted in extension of the range of attractiveness from between 3–7 m to between 7–11 m; rates of 500 and 250 ml/min did result in an increase in number of mosquitoes in the traps. Correspondingly, significantly more mosquitoes were caught in the traps at 3 m when the rate was increased to 1,000 ml/min from 500 ml/min. For *Ae. vexans*, 4,000 ml/min of CO<sub>2</sub> attracted more mosquitoes to the 7 m traps than 1,000 ml/min. In this work carbon dioxide did not result in an increase in the number of *Culiseta inornata*, *Cs. morsitans* and *Culex restuans* and *Cx. pipiens* in the traps.

## INTRODUCTION

Previous field studies on African (Gillies and Wilkes 1969, 1970, 1972, 1974) and North American (Edman 1979) mosquitoes have investigated the ranges of attraction of animal baits and their carbon dioxide equivalents. Using his own data plus that of Schreck et al. (1972), Gillies (1980) postulated a model whereby the range over which CO<sub>2</sub> was attractive to mosquitoes increased linearly with release rates up to 1,000 ml/min. Notably lacking has been experimental field studies designed to test the effect of release rates on the range of attractiveness to mosquitoes. The purpose of our work was to help correct this deficiency using southwestern Ontario mosquitoes.

## MATERIALS AND METHODS

**Experimental Area:** The experiments were conducted in southwestern Ontario at a location near Aberfoyle (43°34'N, 80°15'W). The experimental site itself was an open field, 90 × 90 m with vegetation primarily grass (*Poa* sp.) and goldenrod (*Solidago* spp.). A mixed deciduous forest occurred around one-half of the perimeter of the site and fields with grass around the other half.

**Experimental Design:** The rationale behind the experimental design used in this work was presented in detail by Gillies and Wilkes (1969). The density of mosquitoes orienting to a bait will rise exponentially as the bait is approached. The distance at which the density starts to rise above the background level will correspond to the most distant point at which mosquitoes detect and respond to the presence of a bait. This point can be readily determined by trapping

mosquitoes at various distances from the bait.

**Traps and Release of CO<sub>2</sub>:** Ramp traps similar in general shape to those of Gillies (1969) were constructed of aluminum window framing and black wire window screen. Trap openings were 1.5 m high by 1 m wide. A catch box (0.25 × 0.3 × 1 m), made of the same material, was located at the top of each ramp. The entrance to the box could be closed by a weighted fiberglass screen door.

In the field, 20 traps were located around a central pressurized CO<sub>2</sub> cylinder; 4 were placed at each of 5 distances (3, 7, 11, 15 and 19 m) from the cylinder. The traps at 3, 11 and 19 m were lined up with their opening to magnetic north, south, east and west. Traps at 7 and 15 m were placed clockwise by the width of one trap in order to make them more accessible to inward flying mosquitoes.

Using a calibrated flowmeter, CO<sub>2</sub> was released at rates of 250, 500, 1,000 and 4,000 ml/min. On control nights the traps were opened but no CO<sub>2</sub> was released. A semirandom release schedule was designed such that on each night in 5 a different flow rate (0–4,000 ml/min) was used, while the order in which rates were used differed between cycles. This was done to overcome any possible bias which might have been caused by experiments with low release rates always following nights with high rates.

Each evening at 1930 hr (EDST), all traps were opened and the flowmeter set to release CO<sub>2</sub> at the appropriate rate. At 0830 hr the following morning, release of CO<sub>2</sub> was terminated and all traps closed. Mosquitoes were removed from the traps using a modified aspirator and taken to the laboratory for identification. Trapping was conducted from 14 June to 15 August 1985 and from 26 May to 11 August 1986. Range of temperature was 20–31°C and wind speed was 0–10 ft/sec in the course of the experiments.

Each day the number of mosquitoes caught in each trap at the 5 distances from the CO<sub>2</sub> source

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was recorded. The geometric mean [Williams' mean (Haddow 1960)] of the catches from the group of 4 traps at each distance from the CO<sub>2</sub> source was calculated for each night's trapping. The arithmetic mean of these geometric means was then calculated, giving a value for the mosquito catch at each distance for each release rate. These values were compared using the computer program for the analysis of variance, SAS PROC ANOVA (SAS Institute, Cary, NC 27511-800) to determine: a) whether catches differed between nights when different release rates were used, for traps at each of the 5 distances, and b) whether catches differed between traps at different distances from the CO<sub>2</sub> source, for each of the 5 release rates.

## RESULTS

Twenty species of mosquitoes were caught in the ramp traps in both 1985 and 1986 as follows: *Anopheles - earlei* Vargas, *punctipennis* (Say), *quadrimaculatus* Say and *walkeri* Theobald.

*Aedes - canadensis* (Theobald), *cinereus* Meigen, *euedes* Howard, Dyar and Knab, *excrucians* (Walker), *fitchii* (Felt and Young), *provocans* (Walker), *punctor* (Kirby), *stimulans* (Walker), *triseriatus* (Say), *trivittatus* (Coq.) and *vexans* (Meigen).

*Culex - pipiens* Linn. and *restuans* Theobald.

*Culiseta - inornata* (Williston) and *morsitans* (Theobald).

*Coquillettidia perturbans* (Walker).

Only *Ae. vexans* and *Ae. walkeri* were trapped in sufficient numbers to allow for statistical analysis on a single species basis. Data for the 2 *Culex* species were grouped for analysis as were those for the 2 *Culiseta* species. The spring *Aedes* complex, that is, *Ae. stimulans*, *Ae. fitchii*, *Ae. euedes* and *Ae. excrucians*, was treated as a group.

For female *Ae. vexans*, catches on nights when the release rate was 4,000 ml/min were significantly greater at 3 and 7 m than on nights when the rate was 1,000 ml/min. In turn, the latter rate attracted significantly more mosquitoes at 3 m than the smaller rates and the control which did not differ from one another (Table 1). A release rate of 4,000 ml/min attracted female *Ae. vexans* from a distance of at least 7 m and a rate of 1,000 ml/min from at least 3 m (Table 1). Results for females of the spring *Aedes* spp. and *An. walkeri* were the same as for female *Ae. vexans*, except that there was no significant difference in the numbers caught at 7 m when the release rate was 4,000 ml/min.

For females of *Culex* spp. and *Culiseta* spp. no significant differences were found between catches at various distances from the CO<sub>2</sub> source or between catches on nights when CO<sub>2</sub> was

released at different rates and catches on control nights (Table 1).

## DISCUSSION

This study demonstrates that for certain mosquitoes, namely *Ae. vexans*, spring *Aedes* spp., and *An. walkeri*, an increase in the release rate of CO<sub>2</sub> from 1,000 to 4,000 ml/min resulted in extension of the range of attractiveness from between 3-7 m to between 7-11 m; rates of 500 and 250 ml/min failed to increase the number of mosquitoes in the traps. Correspondingly, significantly more mosquitoes were caught in the traps at 3 m when the rate was increased to 1,000 ml/min from 500 ml/min. For *Ae. vexans*, 4,000 ml/min of carbon dioxide attracted more mosquitoes to the 7 m traps than 1,000 ml/min. To date it has not been determined in the field if there is a rate of release of CO<sub>2</sub> above which members of a CO<sub>2</sub> responsive species are not attracted from a greater distance or more of them are attracted at a particular distance. Interestingly, in laboratory studies Khan et al. (1967) and McIver (1968) found that an increase in the release rate of carbon dioxide over a critical minimum level does not lead to an increased catch of female *Aedes aegypti* (Linn.).

In view of the findings of Gillies and Wilkes (1972), that *Aedes* spp. and *Anopheles ziemanni* Grünberg were attracted to 500-700 ml/min of CO<sub>2</sub> from at least 15 m, and of Gillies and Wilkes (1970, 1974), that *Anopheles melas* Theobald responded to 50 ml/min from 3 m and 230-330 ml/min from 10 yards (9 m), it seems surprising that the Ontario *Aedes* spp. and *Anopheles* spp. were not attracted from greater distances by the much larger amounts used in our work. This difference in response between African and Canadian species of *Aedes* and *Anopheles* could be due to behavior of the individual species or to wind related effects. Diffusion of a gas is a function of wind speed and turbulence; the latter is largely determined by physical features of the landscape. The experimental sites at Keneba (Gillies and Wilkes 1970, 1972) and Bramaka Bra (Gillies and Wilkes 1974) in Gambia are generally similar enough to our site that differences in turbulence would be small (T.J. Gillespie, personal communication). Wind speeds in the African studies were low, for example, an average of less than 1 ft/sec for several hours (Gillies and Wilkes 1970). Even though the wind speeds in our study were higher, that is, up to 10 ft/sec, this difference does not account for the much greater sensitivity of the African mosquitoes. It seems reasonable to conclude that the greater range of attraction of CO<sub>2</sub> to mosquitoes in the works of Gillies and Wilkes is a function of species rather than of meteorology.

Table 1. Mean catches\* of female mosquitoes in relation to distance from CO<sub>2</sub> source and release rate of CO<sub>2</sub> from 1985 and 1986 field seasons combined.

Species	CO <sub>2</sub> release rate (ml/min)	CO <sub>2</sub> release rate (ml/min)					Total no. trapped
		3 m	7 m	11 m	15 m	19 m	
<i>Ae. vexans</i>	4,000	41.67 (3,827) a	9.33 (806) a	4.71 (364)	3.88 (260)	2.61 (153)	5,410
	1,000	7.33 (616) a	2.37 (133)	1.72 (78)	1.60 (58)	1.41 (37)	922
	500	4.85 (378)	2.13 (93)	1.76 (62)	2.22 (58)	2.06 (54)	645
	250	3.35 (191)	1.85 (65)	1.50 (59)	1.57 (50)	1.76 (60)	425
	0	1.35 (28)	1.29 (23)	1.26 (29)	1.23 (19)	1.23 (19)	118
Total no. trapped	5,040	1,120	592	445	323	7,520	
Spring <i>Aedes</i>	4,000	6.42 (603) a	2.96 (175) a	1.91 (88)	1.99 (95)	1.76 (84)	1,045
	1,000	3.89 (301) a	1.86 (95)	1.55 (60)	1.53 (53)	1.41 (39)	548
	500	1.75 (73)	1.19 (18)	1.18 (17)	1.26 (24)	1.13 (14)	146
	250	2.00 (87)	1.46 (43)	1.32 (34)	1.31 (28)	1.30 (30)	222
	0	1.23 (23)	1.20 (19)	1.18 (18)	1.37 (36)	1.35 (35)	131
Total no. trapped	1,087	350	217	236	202	2,092	
<i>An. walkeri</i>	4,000	20.26 (1790) a	3.77 (236) a	1.57 (55)	1.40 (34)	1.18 (16)	2,131
	1,000	6.76 (526)	1.86 (87)	1.45 (43)	1.19 (18)	1.10 (9)	683
	500	1.77 (83)	1.11 (9)	1.05 (5)	1.04 (5)	1.03 (3)	105
	250	2.52 (125)	1.35 (31)	1.08 (7)	1.09 (8)	1.03 (4)	175
	0	1.01 (1)	1.02 (2)	1.00 (0)	1.02 (2)	1.04 (3)	8
Total no. trapped	2,525	365	110	67	35	3,102	
<i>Culex</i> spp.	4,000	2.35 (120)	2.27 (110)	1.62 (60)	2.20 (100)	1.63 (59)	449
	1,000	1.79 (67)	1.45 (42)	1.38 (37)	1.53 (46)	1.44 (36)	228
	500	1.45 (22)	1.34 (35)	1.27 (27)	1.34 (33)	1.28 (26)	141
	250	1.50 (42)	1.70 (66)	1.43 (38)	1.42 (35)	1.60 (55)	236
	0	1.18 (5)	1.14 (14)	1.23 (24)	1.32 (28)	1.20 (20)	91
Total no. trapped	256	267	184	242	196	1,145	
<i>Culiseta</i> spp.	4,000	1.77 (65)	1.78 (76)	1.69 (62)	1.75 (67)	1.48 (46)	316
	1,000	2.20 (92)	3.34 (73)	2.27 (110)	2.48 (99)	2.19 (114)	488
	500	1.62 (63)	1.52 (60)	1.63 (66)	1.76 (76)	1.71 (82)	347
	250	1.37 (32)	1.32 (31)	1.38 (38)	1.58 (44)	1.55 (55)	200
	0	1.40 (35)	1.42 (42)	1.68 (59)	1.81 (68)	1.80 (75)	279
Total no. trapped	287	282	335	354	372	1,630	

\* Mean catch = arithmetic mean of geometric means of (x + 1) for the 4 traps at each distance from the CO<sub>2</sub> source where x = the number of mosquitoes in each trap; numbers in parentheses are total numbers of female mosquitoes trapped for the 4 traps at each distance, for each CO<sub>2</sub> release rate.

a = Significantly different at 5% level.

In this work, CO<sub>2</sub> did not result in an increase in the number of *Cs. inornata*, *Cs. morsitans*, *Cx. restuans* and *Cx. pipiens* in the traps. Other *Culex* species have exhibited various responses to CO<sub>2</sub>. At one West African site *Culex decens* Theobald responded to CO<sub>2</sub> released at a rate of 230–330 ml/min from a distance of at least 5 yards (4.6 m) (Gillies and Wilkes 1970), while at another site the same species did not respond to CO<sub>2</sub> presented at 500–700 ml/min (Gillies and Wilkes 1972). A 230–330 ml/min release rate of CO<sub>2</sub> attracted *Cx. tritaeniorhynchus* Giles and *Cx. thalassius* Theobald from at least 10 yards (9 m), and a rate of 300–700 ml/min caused a convergence of the latter species and *Cx. univittatus* Theobald towards the bait from

at least 7.5 m (Gillies and Wilkes 1970). In Florida Edman (1979) found that *Cx. nigripalpus* Theobald responded to 75–80 ml/min from a distance of less than 10 m and that *Cx. pilosus* (Dyar and Knab) did not show any appreciable orientation response to within 5 m. In field studies in California *Cx. tarsalis* (Coq.) was found to respond positively to CO<sub>2</sub> (Reeves 1951, 1953).

From our results and those of Edman (1979) and of Gillies and Wilkes (1969, 1970, 1972), the responses of *Culex* spp. to CO<sub>2</sub> appear to be weak in comparison to those of *Aedes* spp. and *Anopheles* spp. In general, *Culex* spp. are ornithophilic, and *Aedes* spp. and *Anopheles* spp. are mammalophilic. The difference among the genera is

not a function of various levels of CO<sub>2</sub> production by mammals and birds, because the levels of CO<sub>2</sub> tested, especially the higher ones in this study, are several times greater than the rate of release by even large hosts; for example, Hereford beef heifers release CO<sub>2</sub> at rates from 1,200 to 1,800 ml/min (Roberts 1972), and 5 small chickens release CO<sub>2</sub> at 50 ml/min (Gillies and Wilkes 1974). As suggested by Gillies and Wilkes (1970, 1972), many *Culex* spp. must rely on host-associated olfactory cues in long range orientation rather than CO<sub>2</sub>. *Culiseta inornata* which prefers to feed on wild and domestic mammals (Steward and McWade 1961) and *Cs. morsitans* which is primarily ornithophilic (Morris et al. 1976) but also bites small mammals and snakes (Hayes 1961) must place reliance on host-associated odors in long range orientation as well.

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