CLASSIC PAPER

QUANTITATIVE FIELD STUDIES ON A CARBON DIOXIDE CHEMOTROPISM OF MOSQUITOES[1]

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The host preferences of different species of mosquitoes, which are relevant to the epidemiology of diseases carried by these vectors, have long been recognized and long unexplained. A previous study (Reeves 1951) confirmed that various mosquito species were attracted to carbon dioxide gas. This finding suggested that a carbon dioxide chemotropism may be a major factor in host selection. Investigations of the role of mosquitoes in the epidemiology of the arthropod-borne viral encephalitides in Kern County, CA, in 1951 showed the practicability of studies on the quantity of carbon dioxide preferred by different species of mosquitoes. A more extensive, more precise study was therefore undertaken and forms the basis of the present report.

In general, the procedure was to provide concentrations of carbon dioxide representing approximately the amount excreted by the chicken, man and the horse (or cow) and then to find out the number and species of mosquitoes that found these baits attractive.

MATERIALS AND METHODS

Two stable traps of the design described by Bates (1944) were the standard apparatus for mosquito collection. Tanks of compressed carbon dioxide of 25 lb (6,000 liter) capacity were the source of gas. The volume released was controlled by a gas pressure regulator with a gauge dial calibrated in pounds per square inch. The final release was through the orifice of a glass capillary tube with the tip encased in heavy rubber tubing to protect it from breakage. The glass capillary tubes were prepared and calibrated by measuring the flow of carbon dioxide in milliliters per minute that passed through a wet gas meter at various differential pressures. Four such tubes were used: two covered the range of flow of 20–300 ml/min, and two the range 600–2,500 ml/min.

Three rates of release were selected for these studies on the basis of various published estimates: 25 (chicken), 250 (man) and 2,500 ml/min (horse or cow). Because of the lack of sensitivity of the pressure gauge, it was not possible to maintain the flow precisely at these levels for 12 h or longer. Therefore, at the beginning of a trial the apparatus was set to release slightly more than the desired amount, and by the termination of the trial the amount being released had decreased to a level below that selected. Readings were taken at the beginning and end of each trial, and the range and average flow were computed. The mean actual flow is presented in the tables; it was usually below the selected level. In the discussion of the results, to simplify the discussion, the 3 levels—25, 250 and 2,500 ml—will be used to represent the ranges studied. All trials in which the equipment failed to function satisfactorily were excluded, regardless of the number of mosquitoes collected.

On each test, 2 traps spaced approximately 40

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Dr. Reeves' paper established unequivocally that carbon dioxide is attractant to mosquitoes in the field, effectively ending a long period of controversy on the question. Beyond that, this paper demonstrated that carbon dioxide chemotropism is a matter of fundamental importance in the chemical ecology of mosquitoes and the transmission of mosquito-borne diseases and, in addition, introduced a major advance in then-existing techniques for collecting mosquitoes and monitoring mosquito populations.—L. C. Rutledge.

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ft apart were run simultaneously. When the attractiveness of 2 flows was being compared, the different flows were alternated between the 2 traps on successive nights. This decreased the chance that average collection figures would be affected by location of the traps, rather than by differences in the attractiveness of gas concentrations.

The 250 ml flow was selected as the constant one; it was compared with 25 and 2,500 ml. The latter two were never compared directly.

Tests were made in 2 areas: the first was on the banks of a natural watercourse known as Poso Creek. Conditions in this stream were favorable for development of aquatic stages of *Culex tarsalis*, and large numbers were present along its margins throughout the study. As a result, the population of *Cx. tarsalis* adults was very large. Only small numbers of other species inhabited the area. The traps were placed in the shade of trees on the creek bank within 50 ft of breeding areas and adult resting shelters. The second area, Palm Ranch, was a large ranch on the margin of an arid region. A number of different species of mosquitoes were to be found in the area, probably because the water sources, which resulted from irrigation, were of diverse types. The 3 principal species were *Cx. tarsalis*, *Cx. quinquefasciatus* and *Aedes nigromaculis*. In this area the traps were placed in an open unshaded pasture on which several hundred cattle were feeding.

Trials were made on 20 nights. Most of them were begun between 1700 and 1800 h when the gas was turned on and the flow regulated. They were terminated between 0800 and 0900 h the following morning when the gas was turned off and all mosquitoes in the traps were collected in cages and taken to the laboratory for identification and counting.

At Palm Ranch, because of its distance from the field station and time taken to get there, in trials at the 2 lower levels (25 and 250 ml), the gas was allowed to run continuously day and night. Mosquitoes were removed each morning between 0900 and 1000 h, and at the same time cylinders, with gauges and capillary tubes attached and running, were interchanged between traps. In trials with 2,500 ml it was necessary to limit runs to 16-h periods or less because of the short life of cylinders at this flow (only 3 runs).

RESULTS

Because of the difference in the size and species of the mosquito populations in the 2 study areas, each area is considered separately. All collection figures refer only to female mosquitoes; only 22 males (all *Cx. tarsalis*) were collected.

Poso Creek: A total of 5,443 mosquitoes was collected in this area in 18 trials (Table 1). *Culex tarsalis* was the only species trapped in numbers adequate to permit comparison of the attractiveness of the different concentrations of carbon dioxide. Progressively larger numbers of this species were attracted as the rate of flow of carbon dioxide was increased; even the smallest amount attracted large numbers.

Consideration of the specific tests in which the effects of 2 different rates of flow were successfully compared on the same night shows that 250 ml attracted large numbers when it was competing with 25 ml, but much smaller numbers when competing with 2,500 ml (Table 2).

Palm Ranch: A total of 2,821 mosquitoes were collected in this area in 15 trials (Table 3). *Culex tarsalis*, *Cx. quinquefasciatus* and *Ae. nigromaculis* were collected in numbers sufficient to allow analysis. The number of *Cx. tarsalis* again increased as the flow of carbon dioxide was increased. The trend was quite similar to that at Poso Creek, even though the total population of this species at Palm Ranch appeared to be much less. At the higher rates of carbon dioxide release the number of *Cx. quinquefasciatus* collected decreased. *Aedes nigromaculis* and possibly *Ae. dorsalis* were attracted in the largest numbers to the highest rate of release; practically none were collected at the 25 ml level.

In specific comparative trials (Table 4), the number of *Cx. tarsalis* collected in 250 ml tests was higher when this bait competed with the 25 ml rate than when it competed with the 2,500 ml rate. The same may be true for *Cx. quinque-*

Table 1. Species and number of female mosquitoes collected in trials with different flows of carbon dioxide, Poso Creek area.

Mean flow of CO_2 (ml/min)	No. of trials	*Cx. tarsalis* Total	*Cx. tarsalis* Mean per trial	*Cx. quinquefasciatus* total	*Cx. thriambus* total	*Ae. nigromaculis* total	*Ae. franciscanus* total	All species Total	All species Mean per trial
24	5	863	173	4	1	0	0	868	174
241	11	3,069	279	25	1	5	0	3,100	282
2,475	2	1,453	727	1	0	8	13	1,475	738

fasciatus, but not for the 2 species of *Aedes* collected.

DISCUSSION

The attractiveness of different environmental concentrations of carbon dioxide to different species of mosquitoes may now be said with assurance to have biological implications. Present data, although limited, reveal differences of a magnitude justifying more extensive study. The tendency of the number of attracted *Ae. nigromaculis* to increase when the concentration of carbon dioxide is higher, in contrast to the tendency of the numbers of *Cx. quinquefasciatus* to decrease (Fig. 1), fits the field observations on their host preferences. It is possible that *Ae. nigromaculis* is not attracted to birds because this mosquito may not be able to detect the relatively small amounts of carbon dioxide released by chickens and smaller birds, and finds them only occasionally and by accident in a random search for hosts. As concentrations of the stimulant are increased, as though by larger hosts, the range of detection is entered and the hosts are easily found; the optimum range may be the amounts of this gas given off by larger animals.

In contrast are the reactions of *Cx. quinquefasciatus* and *Cx. tarsalis* (Fig. 1). The relatively lower concentration of carbon dioxide given off by avian hosts may have the maximal attractiveness for the first of these 2 species, which apparently is attracted less as concentrations increase. A pattern of this type would be expected of an aviphilic species.

Culex tarsalis reacted differently than was ex-

Table 2. Species and number of female mosquitoes collected in trials comparing the attractiveness of 2 different flows of carbon dioxide on the same night, Poso Creek area.

Mean flows of CO_2 compared (ml/min)	No. of trials	Mosquitoes collected: *Cx. tarsalis* Total	*Cx. tarsalis* Mean per trial	Total other species
26	4	665	166	3
224		980	245	13
235	2	332	166	6
2,500		1,453	727	26

Table 3. Species and number of female mosquitoes collected in trials with different flows of carbon dioxide, Palm Ranch area.

Mean flow of CO_2 (ml/min)	No. of trials	*Cx. tarsalis* Total	*Cx. tarsalis* Mean per trial	*Cx. quinquefasciatus* Total	*Cx. quinquefasciatus* Mean per trial	*Ae. nigromaculis* Total	*Ae. nigromaculis* Mean per trial	*Ae. dorsalis* Total	All species Total	All species Mean per trial
32	3	280	93	77	26	5	2	0	362	121
242	8	1,110	139	174	22	140	18	9	1,433	179
2,425	4	664	166	49	12	301	75	12	1,026	257

Table 4. Species and number of female mosquitoes collected in trials comparing the attractiveness of 2 different flows of carbon dioxide on the same night, Palm Ranch area.

Mean flows of CO_2 compared (ml/min)	No. of trials	Mosquitoes collected: *Cx. tarsalis* Total	*Cx. tarsalis* Mean per trial	*Cx. quinquefasciatus* Total	*Cx. quinquefasciatus* Mean per trial	*Ae. nigromaculis* Total	*Ae. nigromaculis* Mean per trial	*Ae. dorsalis* Total
32	3	280	93	77	29	5	2	0
250		459	153	104	35	6	2	1
238	4	498	125	56	14	54	14	7
2,400		664	166	49	12	301	75	12

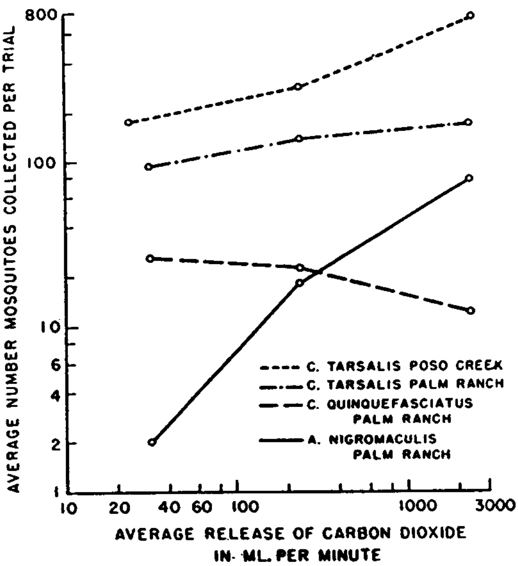

Fig. 1. Average number of mosquitoes attracted by different flows of carbon dioxide.

pected; large numbers were attracted to all 3 levels of carbon dioxide, and the numbers collected increased as the level increased. Former observations had indicated that this mosquito is mainly aviphilic, feeding most frequently on birds, but it is also known to feed commonly on large mammals. If this species is equally or more attracted to the greater concentrations of carbon dioxide, one might question its aviphilic tendencies or the specificity of this stimulant. However, further consideration of environmental factors may resolve this dilemma. In the areas where the host preference tendencies of *Cx. tarsalis* have been discerned, the populations of wild and domestic birds have been very large. Avian hosts far outnumbered large mammals and were therefore more available. Under these circumstances, in a search for blood, a species of mosquito capable of detecting low concentrations would have a much better chance of finding an avian source than a large mammalian source of blood. This would be particularly true if their range of detection is short.

On entering traps to remove mosquitoes, the collector was attacked quickly unless mosquito repellent was used. *Culex tarsalis* seldom bites in the daytime, but under the conditions of these experiments it seemed unduly stimulated. It was most common to observe mosquitoes probing at the rubber tubing where gas was released as if they were in search of blood.

The present data are too limited to permit more than an observation of trends and the drawing of inferences. Additional studies are needed in a greater variety of environmental situations, particularly with different sizes and species of mosquito populations and with other amounts of carbon dioxide.

The phenomenon of interference of one level of stimulant with another is of interest because of its possible role in ecological situations where more than one host species is present and is therefore possibly in competition with other hosts in the attraction of mosquitoes. The number of *Cx. tarsalis* attracted when the rate was 250 ml increased in both study areas after the alternate rate of 2,500 ml was replaced by 25 ml (Tables 2 and 4). This phenomenon could be explained either by competition between the 2 attractants or an increase in mosquito population at this time. One might question whether the mosquito population would increase coincidentally in 2 widely separated areas but, of course, it is a possibility. (Competition of hosts is reminiscent of the efforts to employ large domestic animals as a protective barrier for man in malaria control.)

It cannot be concluded that carbon dioxide is more attractive to *Cx. tarsalis* than to the other species collected because the population of this species in the 2 areas was probably larger. *Culex tarsalis* is, however, the one species sufficiently common in both areas to permit comparison of the attractant. Figure 1 illustrates that trends in the number of specimens attracted at the 2 lower rates of carbon dioxide release ran parallel in spite of the differences in the size of the mosquito population in the 2 areas. The relationship diverges at the higher rate of stimulant release, although in both areas the number of mosquitoes was higher than at the lower rates tested. This divergence could well be due to factors such as population pressure, competition of other attractants or variability in data due to the small number of tests run.

Development of a technique for sampling the population of mosquitoes preferring a certain host may be possible by using the principle of releasing a quantity of carbon dioxide comparable to that given off by the host in question. In investigations of mosquitoes suspected as disease vectors, such a sampling technique could be of considerable epidemiological value. The recent Canadian studies (Brown 1951) of factors attracting *Aedes* mosquitoes to man included this possibility by releasing from robots carbon dioxide in amounts approximating that released by man. The findings of this study appear to be consistent with those of the present investigation.

SUMMARY

Carbon dioxide, released at rates of approximately 25, 250 and 2,500 ml/min, was successfully employed as a host-simulating mosquito

attractant. These rates were selected because they represent approximately the average amounts of carbon dioxide given off by chicken, man, and horse or cow, respectively.

Large numbers of female mosquitoes of several species were attracted into traps baited with these concentrations of carbon dioxide. The various species of mosquitoes responded differently. *Aedes nigromaculis* was attracted in the greatest numbers to the higher concentrations. More *Cx. quinquefasciatus* were attracted to the least amount. *Culex tarsalis* was greatly attracted to all 3 concentrations, but the largest numbers were attracted to the traps where the rate of release was highest. These observations are correlated with available knowledge of the host preference habits of these species, and they suggest a direct interrelationship of host-seeking and carbon dioxide chemotropism.

These observations are of potential value in epidemiological studies of mosquito-borne diseases and offer a new investigative tool in such research.

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