

Airspray applications in the Great Basin are subject to the vagaries of mountain winds. More than two-thirds of a series of carefully planned test plots had to be applied in winds exceeding 4 miles per hour. Little difference was found in the penetrating abilities of sprays applied at 1 gallon, 1 quart, and 5.2 fl. oz. per acre. The authors suggest that, while more susceptible to drift, rates under one-half gallon per acre may be used in areas away from agriculture in order to treat more acreage in the limited periods of minimum wind conditions that are common to mountainous areas (Burgoyne and Akesson 1968).

References

AKESSON, N. B. and YATES, W. E. 1967. Criteria for minimizing the hazard of drift from aerial applications. Proc. 60th Annual Meeting of the American Society of Agricultural Engineers; Saskatoon, Saskatchewan.

ANDERSEN, D. M. 1966. Ecological factors affecting mosquito control on Utah marshes. Doctoral Thesis, University of Utah, June.

BEHLE, W. H. 1969. Syllabus for Wildlife Ecology. University of Utah Press.

BURGOYNE, W. E. and AKESSON, N. B. 1966. A low volume spray system for small aircraft used in mosquito control. Calif. Vector Views 13(8):63-66.

BURGOYNE, W. E. and AKESSON, N. B. 1968. Aircraft operations in the Colusa Project. DOWN TO EARTH, Dow Chemical Co., Midland, Michigan; Fall 1968:7-10.

COLLETT, G. C. 1968. Summary of the Thirty-Ninth Annual Report of the Salt Lake City Mosquito Abatement District.

MAKSYMUK, B. 1964. The drop size method for estimating mass median diameter of aerial sprays. U. S. Forest Service Research Paper WO-1, Forest Insect Laboratory, Beltsville, Maryland. April.

REES, D. M. 1968. Mosquito control developments in Utah and vicinity. Proc. of the 36th Annual Conference of the Calif. Mosquito Control Association.

U. S. Department of Commerce (Weather Bureau) 1969. Local climatological data, Salt Lake City International Airport, April through September 1969.

ANALYSIS OF CO₂ SUPPLEMENTED MOSQUITO ADULT LANDING RATE COUNTS

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The ability to accurately sample an adult mosquito population in the field has always been a problem for mosquito workers, both in research and control. Huffaker and Back (1943) covered this subject well when they stated "the many established methods of sampling have been severely and justifiably criticized on the grounds of the selectivity of the methods."

The aim of this study was to provide organized mosquito control districts with

a surveillance method, not dissimilar to the methods in practice, but one which would more accurately sample the population over the entire day of inspection and with little regard for the variables produced by higher midday temperatures and greater light intensities.

The authors, Harden and Poolson (1969), in preparing a previous paper on seasonal distribution of mosquitoes in Southern Mississippi encountered these very problems even when using several methods (e. g. New Jersey light traps, CO₂ supplemented CDC miniature light traps, daytime landing rate counts, truck traps and larval sampling). There was

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not a single consistent method, except perhaps CDC light traps with CO₂; however, these required overnight operation which does not give an on-the-spot evaluation. At this point we decided to use daytime adult landing rate counts with dry ice furnishing a CO₂ supplement. As a result, beginning in the spring of 1969, a field study was initiated simultaneously with a literature review. The results of the literature search were as follows:

The use of CO₂ as a stimulus or attractant for mosquitoes has been known for some time. As early as 1922, Rudolphs suggested the use of CO₂ to activate mosquitoes. Headlee, in 1933, first used CO₂ to supplement New Jersey light traps and found that collections were increased 400 to 500 percent. Reeves (1951-53) carried on extensive studies using CO₂ as a supplement for mosquito light traps. He felt that CO₂ chemotropism of mosquitoes may be the major factor in host selection and that there is attractiveness of different environmental concentrations of CO₂ to different species of mosquitoes. For example, he found that in CO₂ baited light traps, *Aedes nigrimaculatus* were attracted in greatest numbers to the highest concentrations of CO₂. *Culex quinquefasciatus* were attracted in greatest numbers to the lowest concentration, while progressively larger numbers of *Culex tarsalis* were attracted as CO₂ flow rates were increased.

Huffaker and Back (1943) found that there is evidence that males are repelled by CO₂. Brown *et al.* (1951) concluded that CO₂ may *not* be an attractant but an activator; whereas, Kahn and Maibach (1966) felt that carbon dioxide plays a dual role—a primary role of activation of mosquitoes and a secondary role as inducing landing and probing. Studies by Newhouse *et al.*, (1966) and Carestia and Savage (1967) indicate that the number of varieties of mosquitoes collected in light traps baited with CO₂ were significantly greater than those unbaited. Whitsel and Schoepner (1965) found that the amount of CO₂ released from a pound of dry ice is equivalent to that discharged by 8 to 12

humans. Encouraged with these data to reinforce our approach, initial sampling was begun in May, 1969, and concluded in November, 1969.

PROCEDURE. It was immediately evident that mouth aspirators, chloroform tubes, and flashlight type auto-vacuum cleaners converted into aspirators were not adequate for quick and easy collection of all mosquitoes landing on a collector within a limited time period.

Fortunately, the City of New Orleans Mosquito Control District had developed a 12-volt portable automotive vacuum cleaner into a highly efficient mosquito collecting device. One-inch plastic tubing was adapted to screw onto the suction tube, with a mosquito netting seal on one end and a cork stopper on the terminal end. A surplus metal ammunition case was utilized to carry two 6-volt dry cell batteries adapted to an automotive cigarette lighter unit into which is plugged the vacuum unit. (See Figure 1). This unit can actually capture mosquitoes in mid-air. Cost of unit is less than \$10.00 plus batteries.

In order to determine an adequate collection time, a 15-minute sample collected in three 5-minute increments, was made utilizing approximately 3 pounds of dry ice placed in a perforated bucket within 2 feet of the collector. Seventy-five replications yielded 19 species (See Table 1) with a total of 2,823 specimens. The results were as follows: First 5 minutes—35 percent of the total collection; second 5 minutes—31.8 percent and 33.2 percent during the third 5 minutes. Based on these results, all collections made during the balance of the summer were for 5 minutes. Nine of nineteen species had 40 percent or more of all specimens collected in the first 5 minutes. Floodwater species such as *Aedes canadensis*, *Aedes taeniorhynchus*, and *Aedes sollicitans* had 55 percent and *Aedes infirmatus* had 66 percent collected in the first 5 minutes. *Culex salinarius* were slower, with the three 5-minute periods collecting 16.9 percent, 35.5 percent and 47.6 percent. *Psorophora varipes* were similar, with

26.6 percent, 33.3 percent, and 44.1 percent respectively.

Over a 4-day period, the collections were made at daylight (0600 to 0700) without CO₂ and during 1300 to 1400 hours with CO₂. *Aedes sollicitans* was the only species collected consistently throughout all the plots. For example, during eight replications 100 *Aedes sollicitans* were collected with CO₂ in the morning period with a mean of 12.5 per collection. The same stations collected in the afternoon yielded 104 *Aedes sollicitans* with a mean of 13.0 per collection. Consequently, we may assume, at least with *Aedes sollicitans*, that data collected in the afternoon with CO₂ are quite comparable to those collected at dawn during the crepuscular peak activity period.

During the entire series of 15-minute collections, nine species were collected

with CO₂ and six species without CO₂, an increase of 50 percent. The afternoon collection yielded 50 percent more species. Based on this information, 5-minute collections (comparing ice with no ice) were made throughout the mosquito season in a five-county area, including St. Tammany Parish, Louisiana, and Hancock, Harrison, Jackson, and Pearl River Counties, Mississippi. Twenty-six species were collected, 25 with CO₂, and 18 species without CO₂.

A total of 221 replications of CO₂ versus no CO₂ collections were made as 5-minute collections (See Table 2). A total of 5,285 adults were collected with CO₂; 1,853 without CO₂, with a ratio of 2.8:1 in favor of CO₂.

Reeves (1951) had noted that males of certain *Aedes* were frequently observed in the vicinity of animal hosts, supposedly for the purpose of coming in proximity

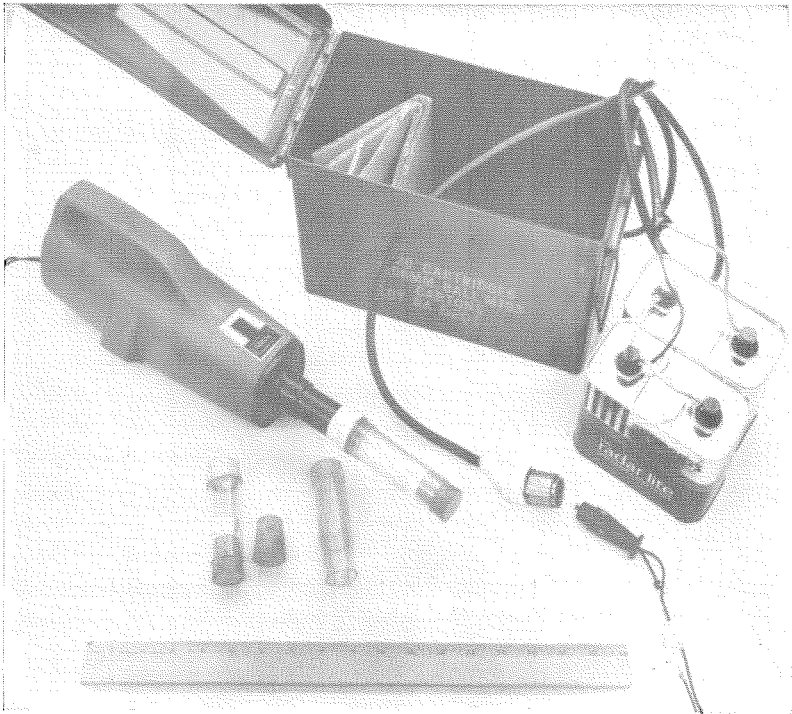


FIG. 1.—Items used in vacuum mosquito collecting device. See text for description.

to females for mating. The authors observed this situation, especially with *Aedes aegypti*. During 11 collections with CO₂, 12 males were collected along with 10 females. *Culex quinquefasciatus* were not collected even though there were high *Culex quinquefasciatus* populations in the area. Special efforts were made, but to no avail, which seemed to support Reeves' data that *Culex quinquefasciatus* are repelled by high CO₂ levels.

It was quite apparent that each species had a different ratio of attractance to a man when supplemented with CO₂. For example, even though the overall ratio of attractance was shown to be 2.8:1, the range varied from 1:1 with *Aedes mitchellae* to 6:1 for *Aedes aegypti* to 8:1 for *Psorophora varipes* and *Culex salinarius*, and to 22:1 for *Aedes vexans*. Eight species were collected only by the CO₂ method. One important factor was the

Table 1. Analysis of 15-Minute Landing Rate Collections Done in 3 - 5 Minute Intervals With CO₂ Supplement.

Species	# Times Collected	Total Mosquitoes Collected	Time Period					
			Five-Minute		Ten-Minute		Fifteen-Minute	
			Total	%	Total	%	Total	%
<i>Aedes atlanticus</i>	29	125	45	36.0	49	39.2	31	24.8
<i>canadensis canadensis</i>	6	17	9	53.0	7	41.2	1	5.8
<i>dupreei</i>	1	1	0	0	1	100.0	0	0
<i>fulvus pallens</i>	1	1	1	100.0	0	0	0	0
<i>infirmatus</i>	6	12	8	66.6	1	8.4	3	25.0
<i>solicitans</i>	33	402	222	55.2	98	24.3	82	20.5
<i>sticticus</i>	7	30	13	43.3	3	10.0	14	46.7
<i>taeniorhynchus</i>	14	18	10	55.5	5	27.7	3	16.8
<i>triseriatus</i>	9	15	5	33.3	5	33.3	5	33.3
<i>vexans</i>	57	979	327	33.4	314	32.7	328	33.9
<i>Anopheles crucians</i>	36	377	164	43.5	124	32.8	89	23.7
<i>quadrimaculatus</i>	6	6	3	50.0	1	16.7	2	33.3
<i>Culex salinarius</i>	41	623	105	16.9	221	35.5	297	47.6
<i>Mansonia perturbans</i>	4	5	2	40.0	2	40.0	1	20.0
<i>Psorophora confinnis</i>	2	2	0	0	1	50.0	1	50.0
<i>ferox</i>	45	202	70	34.7	61	30.2	71	35.1
<i>horrida</i>	1	1	0	0	1	100.0	0	0
<i>howardii</i>	2	2	1	50.0	0	0	1	50.0
<i>varipes</i>	6	15	4	26.6	5	33.3	6	44.1
Total (19)	75	2,623	989	35.0	899	31.8	935	33.2

Table 2. Comparisons of Landing Rate Collections With CO₂ Supplement vs. Collections Without CO₂ Supplement of 27 Representative Species.

Species	Total Number Replications	# Collected With CO ₂	# Collected Without CO ₂	Mean With CO ₂	Mean Without CO ₂	5-Minute Maximum Collected		Ratio CO ₂ vs. Without CO ₂	# Negative Collections With CO ₂	% Negative With CO ₂	# Negative Collections Without CO ₂	% Negative Without CO ₂
						With CO ₂	Without CO ₂					
<i>Aedes aegypti</i>	5	6	1	1.2	0.2	9	1	6.0:1	0	0	4	80
<i>atlanticus</i>	40	342	197	8.55	4.9	75	60	1.7:1	6	15	18	45
<i>canadensis</i>	11	16	5	1.45	0.45	4	2	3.2:1	0	0	7	63
<i>dupreii</i>	2	2	0	1.0	0	1	0	---	--	--	--	--
<i>fulvus pallens</i>	*1 3	*2 3	--	1.0		*1	--	---	--	--	--	--
<i>infirmatus</i>	27	40	12	1.5	0.44	6	3	3.4:1	4	15	17	63
<i>missillae</i>	3	2	2	0.67	0.67 (3)	1	1	1:1	0	0	1	33
<i>pollicitans</i>	131	2,630	1,393	20.1	10.6	197	128	1.9:1	6	4	26	20
<i>sticticus</i>	2	0	2		1.0	0	1	---	--	--	--	--
<i>sticticus</i>	*1 5	*2 25	--	5.0	--	12	--	---	--	--	--	--
<i>taeniorhynchus</i>	45	65	52	1.4	1.2	9	8	1.2:1	15	33	18	40
<i>thibaulti</i>	1	0	1	0.0	1.0	0	1	---	--	--	--	--
<i>triseriatus</i>	19	23	3	1.2	0.16	3	2	7.5:1	1	5	17	89
<i>weesei</i>	114	900	42	7.9	0.37	74	5	22:1	0	0	89	77
<i>Anopheles crucians</i>	74	535	41	7.2	0.58	55	18	13:1	0	0	58	78
<i>punctipennis</i>	2	2	0	1.0	0.0	1	0	---	0	0	2	--
<i>quadrimaculatus</i>	15	39	2	2.6	0.13	11	1	19.5:1	0	0	13	86
<i>Culex salinarius</i>	76	388	50	5.1	0.66	43	12	7.8:1	2	2	58	76
<i>Culiseta inornata</i>	11	16	0	1.45	0.0	3	0	---	0	0	11	100
<i>Mansonia perturbans</i>	3	3	0	1.0	0.0	1	0	---	0	0	3	100
<i>Pedrophora ciliata</i>	2	5	3	2.5	1.5	4	2	---	--	--	--	--
<i>confinis</i>	4	3	3	0.75	0.75	2	2	1:1	2	50	2	50
<i>cyanocephala</i>	1	0	1	0.0	1.0	0	1	---	--	--	--	--
<i>ferox</i>	66	259	42	3.8	0.64	44	15	6:1	4		47	71
<i>horrida</i>	*1 3	*2 3	--			*1	--	---	--	--	--	--
<i>howardii</i>	1	1	0	1.0	0.0	1	0	---	--	--	--	--
<i>varipes</i>	6	6	1	1.3	0.17 (4)	3	1	8:1	0		5	83
	221	5,285	1,853					2.8:1				

NOTE: *1 - Data includes collections with CO₂ only.
 *2 - Data not included in total mosquitoes.
 () - Those collected by CO₂ only without a no CO₂ comparison.

percentage of negative counts without CO₂ compared to the positive collections while using CO₂. In other words, without CO₂, we would have completely missed 30 percent or eight species, and also 89 percent of *Aedes triseratus*, 86 percent of *Anopheles quadrimaculatus*, 80 percent of *Aedes aegypti*, 78 percent of *Anopheles crucians*, 77 percent of *Aedes vexans*, and 63 percent of *Aedes canadensis* and *Aedes infirmatus*.

When comparing simultaneous counts with CO₂ against counts without CO₂, 60 percent of the time the CO₂ supplement counts were positive, while counts without CO₂ were negative.

The authors admit that there is a great deal to be learned about using CO₂ to supplement adult landing rate counts, especially in the interpretation of the results for various species; however, we feel it has the potential of being one of the most reliable sampling methods available to mosquito control workers.

Literature Cited

BROWN, A. W. A., SARKARIA, D. S. and THOMSON, R. P. 1951. Studies on the responses of the female *Aedes* mosquito. Part I—The search for attractant vapours. Bulletin Entomological Research 42.

CARESTIA, R. R. and SAVAGE, L. B. 1967. Effectiveness of carbon dioxide as a mosquito attractant

in the CDC miniature light trap. Mosquito News 27(1):90-92.

HARDEN, F. W. and POOLSON, B. J. 1969. Seasonal distribution of mosquitoes of Hancock County, Mississippi 1964-68. Mosquito News Vol. 29(3), 407-414.

HEADLEE, T. J. 1934. Mosquito work in New Jersey for year 1933. Jour. Proc. 21st Annual Meeting New Jersey Mosq. Ext. Assoc. pp. 8-37.

HUFFAKER, C. B. and BACK, R. C. 1943. A study of methods of sampling mosquito populations. Jour. Econ. Ent. Vol. 36(1), 561-569.

KHAN, A. A. and MAIBACK, H. I. 1966. Quantitation of effect of several stimuli and landing and probing by *Aedes aegypti*. Jour. Econ. Ent. Vol. 59(4), 902-905.

NEWHOUSE, V. F., CHAMBERLAIN, R. W., JOHNSON, J. G. and SUDIA, W. D. 1966. Use of dry ice to increase mosquito catches of the CDC miniature light trap. Mosquito News Vol. 26(1), 30-35.

REEVES, W. C. and HAMMON, W. C. 1942. Mosquitoes and encephalitis in the Yakima Valley, Washington. IV, a trap for collecting live mosquitoes. Jour. Inf. Dis. Vol. 70, 275-277.

REEVES, W. C. 1951. Field studies on carbon dioxide as a possible host stimulant to mosquitoes. Proc. Soc. Exp. Bio. and Med. Vol. 77, 64-66.

REEVES, W. C. 1953. Quantitative field studies on carbon dioxide chemotropism of mosquitoes. Am. Jour. of Trop. Med. and Hygiene. Vol. 2, 325-331.

RUDOLFS, W. 1922. Chemotropism of mosquitoes. Bull. N. J. Agric. Exp. Station, No. 367, 23 pp.

WHITSEL, R. H. and SCHOEPPNER, R. F. 1965. The attractiveness of carbon dioxide to female *Leptocnops torrens* and *L. kerteszi*. Mosquito News, Vol. 25(4), 403-410.

ANNOUNCEMENT . . .

Genetic Markers and Incompatible Strains of *Culex pipiens*
Available Through U.S.-Japan Cooperative Medical Science Program

The National Institute of Allergy and Infectious Diseases, National Institutes of Health has arranged through a contract with the University of California, Los Angeles for the maintenance and supply of several strains of *Culex pipiens*. These strains are useful for studies of cytoplasmic incompatibility, formal genetics and susceptibility to filarial parasites.

Individuals interested in further information concerning these strains and markers should contact

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