Observations on Malathion-resistant Adults of *Anopheles albimanus* Wiedemann in Coastal El Salvador *

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During the dry season of 1969, the Central America Malaria Research Station (CAMRS) conducted field trials in coastal El Salvador to evaluate the efficacy of aerially applied ultra-low-volume (ULV) malathion in reducing populations of *Anopheles albimanus* Wiedemann. Erratic results in mortalities obtained between caged mosquitos from wild and colony sources and between wild populations from different localities prompted investigations into the possible presence of malathion resistance in the species. According to Georghiou (1969), "no serious case of resistance" to organophosphorus insecticides has been previously reported in anopheline mosquitos.

This note summarizes results from laboratory and field data that indicate the presence of malathion resistance among *An. albimanus* at a level sufficient to reduce the effectiveness of field applications of ULV malathion in coastal El Salvador.

Methods

Field studies. ULV trials were conducted in selected coastal areas of El Salvador between Barra de Santiago, near the Guatemala border, and Estero San Diego, about 8 km east of the port and city of La Libertad. All treatments were made by C-47 commercial aircraft flying at an altitude of 125 feet (38 m) and a speed of 160 mi/h (260 km/h). A total of 10 tests was conducted between 18 February 1969 and 5 May 1969. Treatments, made at dawn, were at the rate of 3.0 US fl oz of technical malathion per acre (219 ml/ha). Each treatment involved evaluation by use of strategically placed platform stations including some combination of (*a*) malathion-sensitive oil-red dye cards, (*b*) caged female mosquitos, and (*c*) containers of larval mosquitos. Test specimens were exposed for the duration of the treatment, approximately 1 hour, after which an additional 45 minutes was allowed for complete settling of droplets. In addition to the platform stations, pre- and post-treatment counts using various indices (light-traps, human and animal bait, natural resting, etc.) were made in each area subjected to treatment. Thus, mosquito mortality, or population changes, at a given index station could be equated with malathion droplet distribution on associated dye cards. Collectively, data from these stations revealed the extent of coverage in terms of insecticide distribution and effectiveness.

Laboratory studies. Discrepancies in field mortalities between caged colony and caged wild-caught mosquitos and among wild-caught mosquitos from different localities, when measured against malathion droplet distribution, led to laboratory tests to measure the malathion susceptibility of mosquitos from different sources.

All tests were conducted in accordance with WHO methods in the CAMRS laboratory where the ambient temperature ranged from 24°C to 29°C and the relative humidity from 42% to 63%. Comparative observations were made with adult *An. albimanus* from the CAMRS colony.

Initially, WHO malathion test papers were not available when a quick indication of *An. albimanus* susceptibility to malathion was needed. Thus, a bioassay was conducted by preparing a series of test panels (30 cm × 30 cm) constructed of plywood and covered with kraft paper. A series of panels was treated with increasing concentrations of technical reagent-grade malathion in acetone; control panels were sprayed only with acetone. After spraying, the panels were sealed and stored in individual cardboard boxes for 24 hours prior to use. Three WHO bioassay plastic cones attached to each panel were used to expose the mosquitos. *An. albimanus* blood-fed females from colony and field sources were introduced into the cones by aspirator for 60-minute exposures, after which they were removed by carbon

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TABLE 1

24-HOUR MORTALITY OF *ANOPHELES ALBIMANUS* FEMALES FROM COLONY AND WILD SOURCES AND DROPLET DISTRIBUTION OF ULV MALATHION ON ADJACENT DYE CARDS, LA LIBERTAD, EL SALVADOR, 20 MARCH 1969

Station No.	No. of ULV droplets		Colony adults [a]		Wild-caught adults [a]	
	per card	per in² [b]	No. exposed	Mortality (%)	No. exposed	Mortality (%)
1	65	3.3	15	46.6	19	26.3
2	30	1.5	12	66.6	18	11.1
3	600	30.0	18	100.0	19	70.6
4	79	3.9	17	58.8	23	26.0
5	348	17.4	17	94.1	18	33.3
6	103	5.2	19	89.5	28	14.3
7	109	5.5	19	47.4	10	0
8	64	3.2	14	35.7	26	0
9	1 078	53.9	18	88.8	24	4.2
Total	2 476	—	149	71.1	185	19.7
Average	275	13.8	—	—	—	—

[a] Colony females from CAMRS colony; wild-caught females from cattle corral at El Coco within area of treatment. Specimens were exposed to ULV spray in galvanized screen cages (3 in × 2 in × 6 in (7.5 cm × 5 cm × 15 cm)) and then transferred to holding cups for 24 hours, when mortalities were recorded.

[b] 1 droplet per in² = 0.155 per cm².

dioxide knockdown and held in paper cups for mortality counts 24 hours later. The number of mosquitos from each source was approximately equal for each cone and each panel, but the availability of specimens varied with the source and this influenced the number of test mosquitos used.

When WHO malathion test papers became available, standard WHO susceptibility tests were conducted on field-collected blooded females held in the laboratory for at least 16 hours prior to exposure to the insecticide. Females were exposed to malathion-treated papers in WHO test cylinders for 60 minutes, after which they were returned to resting cylinders for mortality counts 24 hours later. Two tests were run as follows: on 21 October 1969, with only three concentrations of insecticide (0.4 ppm, 1.6 ppm, and 6.4 ppm) due to the scarcity of specimens, and on 21 January 1970, with six concentrations (0.4 ppm, 0.8 ppm, 1.6 ppm, 3.2 ppm and 6.4 ppm), when more specimens became available. Appropriate controls were used in each test.

Results and discussion

Although malathion resistance was suspected in earlier ULV trials, the first definitive evidence came as a result of tests conducted on 20 March 1969 near La Libertad, El Salvador. As a part of this treatment, 9 cages of *An. albimanus* adults from the CAMRS colony were directly compared with 9 cages of wild-caught *An. albimanus* obtained from the El Coco cattle corral within the ULV area where resistance was suspected. Both groups of mosquitos were freshly blood-fed and were contained in identical galvanized screen cages on wooden platforms along with a dye card. After exposure to the ULV treatment, the mosquitos were transferred to paper holding-cups. Mortalities were recorded 24 hours later. The results (Table 1) show that at each of the 9 stations the mortality was greater among the colony groups, with over-all mortality of 71.1% in the colony mosquitos and only 19.7% among the wild-caught specimens.

This field evidence of malathion resistance led to laboratory susceptibility tests in which *An. albimanus* females from various sources were exposed to malathion-treated paper-covered panels as previously described. The results (Table 2) show a high level of malathion susceptibility among *An. albimanus* from the CAMRS colony, with 100% mortality at all concentrations. The Acajutla population was the

TABLE 2

SUSCEPTIBILITY TO MALATHION OF *ANOPHELES ALBIMANUS* FROM VARIOUS SOURCES AS DETERMINED BY EXPOSURE TO TREATED PAPER-COVERED PLYWOOD PANELS, 27 MARCH 1969

Source of specimens	No. of specimens per panel [a]	24-hour percentage mortality at concentrations indicated [b]					
		0.4%	0.8%	1.6%	3.2%	6.4%	Control
Acajutla, Hacienda Atalaya (cattle corral)	37	69	84	92	100	100	2
CAMRS colony (Metapán)	29	100	100	100	100	100	3
La Libertad, Estero San Diego (human bait)	22	5	15	29	38	58	3
La Libertad, El Coco cattle corral	21	14	—	30	—	80	5
La Libertad, Hacienda Melara (cattle corral)	11	0	—	9	—	30	0

[a] Total of 3 cones at each concentration.
[b] Specimens exposed for 60 minutes under WHO plastic cones, removed to clean holding cups and held for 24 hours.

next most susceptible, with 100% mortalities being obtained at the 3.2% and 6.4% concentrations. The La Libertad area mosquitos (San Diego, El Coco, Melara) showed lower levels of susceptibility to malathion than did those from the Acajutla area or from the CAMRS colony. From these sources, complete mortalities (100%) were not obtained even at the highest concentration used (6.4%).

Results from standard WHO tests to determine adult susceptibility to malathion reflected the same resistance pattern as previously revealed by field and panel tests. The data in Table 3 show 100% mortalities at 1.6 ppm and higher concentrations among female *An. albimanus* from the CAMRS colony, while this level was reached in only 1 of 4 tests at the highest concentration (6.4 ppm) for female *An. albimanus* from the La Libertad (El Coco cattle corral) area.

While adults from the La Libertad area showed malathion resistance, such resistance in larvae from that area was not observed in the field nor could it be demonstrated in laboratory tests. This aspect requires more investigation and attempts will be made to clarify susceptibility levels of larval populations in the affected areas.

It is significant that the La Libertad area mosquitos were from a part of the coastal plain where cotton cultivation and associated widespread use of multiple types of insecticides is common. On the other hand, the Acajutla area mosquitos, as well as those from the CAMRS colony, were from sources in a part of El Salvador where cotton cultivation,

TABLE 3

RESULTS OF MALATHION SUSCEPTIBILITY TESTS ON ADULT *ANOPHELES ALBIMANUS* FROM A COASTAL AREA OF EL SALVADOR AND FROM THE CAMRS COLONY

Date	Source of females	Percentage mortality at each concentration					
		0.4 ppm	0.8 ppm	1.6 ppm	3.2 ppm	6.4 ppm	Control
21 Oct. 1969	La Libertad, El Coco cattle corral [a]	4 (25)	—	32 (25)	—	100 (23)	4 (25)
	CAMRS colony [a]	28 (25)	—	100 (25)	—	100 (25)	4 (24)
21 Jan. 1970	La Libertad, El Coco cattle corral [b]	16 (74)	21 (49)	33 (69)	64 (64)	92 (77)	9 (68)
	CAMRS colony [a]	17 (20)	38 (24)	100 (25)	100 (24)	100 (22)	8 (25)

[a] Single tests. Figures in parentheses represent the number of females tested.
[b] Three tests at each concentration except at 0.8 ppm (two tests). Figures in parentheses represent the total number of females tested.

and associated insecticide use, is uncommon. The discovery of *An. albimanus* resistance to an organophosphorus insecticide in El Salvador is not surprising when one considers the interrelationships of cotton cultivation, insecticide use and anopheline populations. Cotton production in Central America has increased steadily during the past two decades, having risen from less than 100 000 acres (40 000 ha) and 55 000 bales to a peak of 928 000 acres (376 000 ha) and a reported 1 355 000 bales in the 1964–65 season (Smith & Reynolds, 1968). According to Mobley (1955), almost all of the cotton in Central America is grown on the Pacific plain extending from Tapachula, Mexico, to Guanacaste Province in Costa Rica. This closely approximates to the area that has presented the greatest problem to malaria eradication efforts in the region.

The malathion resistance described in this note was detected among *An. albimanus* in a locality of El Salvador situated within this Pacific coastal plain of heavy cotton production. It is paradoxical that the great increase in cotton production has been attributed to land reform, opening of new areas to agriculture through malaria control and new roads, and government-supported cotton prices (Mobley, 1955).

In El Salvador, many insect pests attack cotton and insecticides are heavily used to improve cotton quality and to increase yield. During the 1967–68 season, El Salvador ranked twenty-third among the world's countries in cotton production, but ranked fourth in yield with 847 kg/ha (Smith & Reynolds, 1968). Such a yield reflects the continuous application of insecticides for six or seven months out of the year, generally between July and January. For a number of years various organophosphorus insecticides, including malathion, have been used against cotton pests. The current recommendations include trichlorfon for the cotton leaf worm and fall armyworm between July and September; parathion-methyl or malathion for the boll weevil from August to the end of the season; and a mixture of parathion and parathion-methyl against the bollworm from September to the end of the season. The number of applications during a season varies. During 1968 and before, the number ranged between 19 and 27 and averaged 23; during 1969, the range was 14–25 and averaged 17 applications. The most frequent applications, at intervals of 4–7 days, come between August and November, corresponding to expected rainy season peaks in *Anopheles* populations.

Since La Libertad area mosquitos had shown resistance to malathion in field treatments and laboratory panel tests, while Acajutla area specimens had shown more susceptibility in the same laboratory panel tests, it is interesting to note the effect of resistance by comparing data from actual ULV treatments conducted in the respective areas. This can be done by comparing data from Tables 1 and 4. Table 1 shows that in the La Libertad (resistant) area, an average of 13.8 ULV droplets per in^2 (2.14 per cm^2) resulted in only 19.7% over-all mortality among caged, locally caught female *An. albimanus* exposed to the treatment. By contrast, Table 4 shows that an average ULV droplet distribution of only 2.4 per in^2 (0.37 per cm^2) resulted in an over-all mortality of 52% of caged mosquitos locally caught in the Acajutla (susceptible) area. Such differences were noted throughout the trials and ultimately all ULV efforts in the La Libertad area were abandoned after it was shown that even doubling the dosage to 6.0 US fl oz of malathion per acre (438 ml/ha) did not significantly improve results.

TABLE 4

24-HOUR MORTALITY OF CAGED *ANOPHELES ALBIMANUS* FEMALES AND DROPLET DISTRIBUTION OF ULV MALATHION ON ADJACENT DYE CARDS, ACAJUTLA, EL SALVADOR, 2 APRIL 1969 [a]

Station No.	No. of ULV droplets		No. exposed	Mortality (%)
	per card	per in^2 [b]		
1	20	1.0	15	26.6
2	18	0.9	16	50.0
3	40	2.0	20	50.0
4	77	3.9	13	38.5
5	27	1.3	20	60.0
6	29	1.5	15	80.0
7	16	0.8	20	25.0
8	102	5.1	20	70.0
9	40	2.0	18	55.5
10	117	5.9	20	60.0
Total	486		177	52.0
Average	48.6	2.4		

[a] Females were captured from stable at Hacienda Atalaya the night before treatment. Specimens were exposed to spray in galvanized screen cages (3 in × 2 in × 6 in) and then transferred to holding cups for 24 hours when mortalities were recorded.

[b] 1 droplet per in^2 = 0.155 per cm^2.

Georghiou (1969) discussed the resistance potential of anophelines and indicated that, to his knowledge, no consistent and *direct* treatment by cholinesterase inhibitors (organophosphorus compounds or carbamates) had been applied against field populations of anophelines, except on a very limited basis. He further pointed out, however, that *indirect* selection has been taking place through the agricultural use of large quantities of organophosphorus compounds and carbamates within the proximity of *Anopheles* breeding areas. He recognized incipient tolerance to malathion in *An. albimanus* in Central America, particularly in Guatemala and Nicaragua, where intense parathion selection pressure is applied for six months each year in the cotton-growing areas.

Anopheles mosquitos inhabiting areas in close proximity to cotton acreage in El Salvador have likewise been subjected to such organophosphorus pressure and the data presented here show a correlation between the cotton-growing areas and resistant populations of *An. albimanus*.

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Susceptibility of *Culex pipiens fatigans* to Fenthion in Rangoon, Burma, 1963–69 *

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The only locality in Rangoon with a history of organophosphorus spraying is Kemmendine. Larviciding with fenthion (OMS-2) emulsifiable concentrate began in March 1966.

The susceptibility of *Culex pipiens fatigans* larvae has been tested in Kemmendine and 9 other localities of Rangoon during the past 6 years. The data were analysed, in 1969, for the entire period, using the computer at WHO headquarters, Geneva, Switzerland, and reference can be made to the WHO Information Circular on Insecticide Resistance [3] for the complete results. The 95% fiducial limits for the LC_{50} and LC_{95} values, and the slope of the probit regression line are given for each larval test.

In this report, the computed LC_{50} and LC_{95} values on the computer print-outs are summarized according to locality and year. A conclusion will be given on whether any appreciable change has occurred in the susceptibility of *C. p. fatigans* during 3½ years of larval control.

Methods

Larval tests. The WHO procedure (WHO Expert Committee on Insecticides, 1963) was used throughout, and fenthion solutions were routinely despatched to Rangoon. The experience gained from many tests showed that 10 concentrations ranging from 0.001 ppm to 0.01 ppm normally provided 3–5 mortality counts between 10% and 90%. Disposable plastic cups holding 250 ml of demineralized water were preferred to glass beakers which required repeated cleaning.

After control in Kemmendine, it was never possible to obtain 3 or more replicates for any test. When larvae became extremely scarce due to progressive reductions in adult density, suitable regression lines were often obtained using concentrations of 0.002, 0.0035, 0.004, 0.0045 and 0.006 ppm.

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[3] *Information circular on insecticide resistance, insect behaviour and vector genetics*, an unpublished WHO document, VBC/IRG/70.9. A limited number of copies of this document is available to persons officially or professionally interested on request to Distribution and Sales, World Health Organization, 1211 Geneva, Switzerland.