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Mosquitoes - Control

INVESTIGATIONS ON CHROMOSOME ARRANGEMENTS, IRRITABILITY AND SUSCEPTIBILITY OF *ANOPHELES ATROPARVUS* AND *A. LABRANCHIAE*[1]

by

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This report covers investigations carried out during the period from July 1959 to December 1960. The subjects studied are as follows:

1. The continuation of the study of chromosome arrangements in anophelines including anopheline colonies submitted to selective pressure by insecticides.

2. The study of irritant effects in anophelines in contact with insecticide-treated surfaces.

3. The study of base-line susceptibility to insecticides of anopheline vectors in Sicily, using and comparing Busvine-Nash and WHO standard methods.

4. The use of all the above methods for the study of resistance and susceptibility to organophosphorus compounds in laboratory colonies of anopheline species and studies on the mechanism of such a resistance.

The strains of *Anopheles* used in these studies were as follows:

A. The Italian strain of *A. atroparvus* originating from Ferrara, Italy. To this colony were added some ovipositions of *A. atroparvus* captured in other areas in 1946, before the beginning of the antimalaria campaign with DDT. This colony has been maintained in our laboratory in Palermo since November 1955.

[1] These investigations have been conducted under a research grant from WHO.

B. The Hamburg susceptible strain, originating from Northern Germany (strain 2) obtained from the Bernhard Nocht Institut für Tropenkrankheiten (Hamburg) on 5 December 1958, and raised thereafter in our laboratory.

C. Strain IV, derived from the Italian strain which had been exposed for 39 generations to the selective action of DDT, both in the larval and adult stages (D'Alessandro & Mariani, 1958).

1. Study of chromosome arrangements in strains of anopheles

Earlier research carried out in this laboratory (D'Alessandro, Frizzi & Mariani, 1957 and 1958) and confirmed by other authors (Mosna, Rivosecchi & Ascher, 1958; Mosna, Palmieri, Ascher & Rivosecchi, 1959) has demonstrated that the pressure of DDT on a larval population of A. atroparvus having heterogeneous chromosome arrangements (standard, inverted homozygous and heterozygous) favoured markedly the survival of individuals with a heterozygous pattern. Further research has led to the following results:

A. In the Italian strain of A. atroparvus, maintained since 1955 in our laboratory at a relatively constant temperature (26°C ± 2°C), a "spontaneous" progressive increase in the number of heterozygotes, which finally reached 50% of the populations, was observed. At this point an equilibrium between larvae with the heterozygote pattern and those with a standard arrangement (about 50% of each) seemed to be established.

B. A similar behaviour was observed in the A. atroparvus strain from Hamburg which has been raised in our laboratory under the same conditions, since December 1958. In fact, although originally (immediately after their arrival here) the colony was made up of 90% standard and 10% heterozygotes, it is at present composed of 57.5% standard homozygotes, 41.5% heterozygotes and 1% inverted homozygotes.

C. The action of DDT does not seem to change the ratio of the standard to the heterozygote in the Italian strain of A. atroparvus after the point of equilibrium between the chromosomal patterns has been reached (50% standard, 50% heterozygote).

On the other hand, with the Hamburg strain the treatment of the larval population with DDT favours the survival of individuals with heterozygous arrangements and makes the rare inverted homozygotes more evident. In fact, while before treatment the chromosomal arrangements are 57.5% standard, 41.5% heterozygote and 1% inverted, after treatment there are 39% standard, 52% heterozygote and 9% inverted.

D. The changes in the percentage ratio of chromosome patterns seem to be accompanied by a reduction in the susceptibility to DDT of the larvae of the Italian A. atroparvus strain. In fact, in experiments done in September 1956 on a larval population of 62% standard, 37% heterozygote and 1% inverted, the concentration of DDT necessary to obtain 50% mortality after 15 minutes' contact[a] was 0.5 p.p.m. However, during tests carried out in October 1960, when the larval population was made up of 46% standard and 54% heterozygote, in order to obtain 50% mortality, it was necessary to use 5 p.p.m. of DDT; that is to say, a concentration 10 times greater.

E. Strain IV, after the twelfth generation, became moderately resistant to DDT; in fact, from the original LC_{50} of 1.6 (adult females), it reached an LC_{50} which varied between 9 and 10%. At the twelfth generation the genetic composition was 21.71% standard, 74.8% heterozygote and 3.49% inverted; it had, therefore, undergone a marked change in comparison with the original composition of 79.1% standard and 20.9% heterozygote and inverted.

After the twelfth generation, selection pressure did not cause any further increase in the resistance of the strain until the thirtieth generation.

At this point, in an attempt to increase the genetic equipment, this strain was crossed with the Hamburg strain, in both directions (♂♂ of strain IV with ♀♀ of Hamburg, and ♂♂ of Hamburg with ♀♀ of strain IV).

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[a] The 15-minute exposure period has been adopted in order to obtain results comparable with those of investigations carried out in 1956 (Bull. Wld Hlth Org. (1957), 16, 859)

At the thirty-second generation (the second after the crossing), the selection of strain IV in the presence of DDT was again studied.

Up to the thirty-ninth generation (the ninth after crossing) no changes in degree of susceptibility to DDT have been observed. However, following cross-breeding, the cytogenetic composition has changed. At present, the old strain IV is made up of 54% standard, 44.5% heterozygotes and 1.5% inverted.

F. Various attempts have been made to obtain a strain composed entirely of individuals with a standard pattern. To this end, isolated egg deposits were raised and those ovipositions which, after an examination of 50% of the individuals, could be presumed to be made up entirely of standard homozygotes, were selected to form a colony. It was possible to observe that strains isolated with the technique described, when examined after several generations, showed the presence of a certain number of heterozygotes.

G. Experiments are now being performed to study the influence of daily temperature changes ($\pm$ 8°C) and salinity on the composition of the strains with regard to the chromosome patterns of the individuals.

The data obtained up to now seem to indicate that in the strains maintained at varying temperatures (26-27°C, day; 18-20°C night), and particularly in water which contained 3-4% NaCl, there is a greater survival of individuals with a standard homozygote pattern. In this way, the strains tend to return to the cytogenetic composition which they had before being raised systematically in the laboratory at a relatively constant temperature and in water containing only a small amount of NaCl.

From results of this group of experiments it appears that under the conditions of the laboratory-breeding of A. atroparvus the individuals with homozygote arrangements (standard and inverted) are more susceptible to unfavourable microclimatic conditions. In fact, in the Italian strain of A. atroparvus composed of 50% standard and 50% heterozygote, on the basis of the probability of combination between individuals with different patterns, an increase in the number of standard and inverted homozygotes should be observed in successive generations. If this does not occur, it is because in such individuals it is likely that there is a higher rate of mortality than that which occurs in individuals with heterozygote patterns. It should be noted that in the crossing over, the invertible segment (zone 44-48 of the left arm of the third chromosome) behaves as an indivisible block. It may be supposed, therefore, that the ratio of the various types of arrangements in the offspring derived from the crossing and recrossing of individuals of standard and inverted homozygote patterns should follow the Mendelian laws. It is, therefore, possible to compare the action of insecticides DDT and dieldrin to all the other conditions which are unfavourable to the life of the larval population of A. atroparvus, and the greater survival of heterozygotes following the pressure of these insecticides can be interpreted as a vigour-tolerance such as is common with hybrids of many species.

Finally, the fact that it has been impossible to maintain a pure colony of A. atroparvus with a standard homozygote arrangement leads to the supposition that the "inverted" mutant can appear spontaneously during the growth of the colony.

2. Studies on the irritant effects in anophelines exposed to surfaces treated with insecticides

For these experiments we have followed the procedure described by Zulueta (1958) using five mosquitos. This procedure corresponds fundamentally to variation (b) of the WHO method (1960), published after our experiments.

We have observed that the following factors influence the results:

(a) nature and intensity of the light source;
(b) the direction of light rays;
(c) temperature and humidity of the environment;
(d) nutritional conditions of the Anopheles.

The most reliable results were obtained using a dark chamber and illuminating the surface under the conical chamber by means of a fluorescent bulb connected to storage batteries ("Pozzo" lamp); this was placed laterally so that the movements of the observer would not cast shadows.

The light intensity was measured with a "Kruss Luxometer" (1 lux = 0.98 lux international = 1.6 lux Helfner = 0.09-foot candle).

TABLE 1. IRRITABILITY TEST

Batches of five mosquitos - Anopheles atroparvus, Italian strain
Conical exposure chambers. Incident light 50 Lux. Temperature 20°-25°C. Relative humidity 58-60%

Females: fasting or 24 hours after blood meal

Serial number	DDT 4.0% Number of take-offs in 15'		Serial number	DDT 8.0% Number of take-offs in 15'		Serial number	Control Number of take-offs in 15'	
	Total	Individual		Total	Individual		Total	Individual
1	120	24	1a	85	17	1b	60	12
2	150	30	2a	75	15	2b	30	6
3	50	10	3a	100	20	3b	20	4
4	70	14	4a	175	35	4b	50	10
5	115	23	5a	165	33	5b	80	16
6	60	12	6a	65	13	6b	10	2
7	85	17	7a	80	16	7b	25	5
8	95	19	8a	135	27	8b	20	4
9	90	18	9a	125	25	9b	60	12
10	125	25	10a	155	31	10b	50	10
11	140	28	11a	180	36	11b	55	11
12	125	25	12a	140	28	12b	45	9
13	120	24	13a	110	22	13b	35	7
14	185	37	14a	185	37	14b	105	21
15	120	24	15a	175	35	15b	60	12
Number of take-offs per mosquito (1650:75) = 22			Number of take-offs per mosquito (1950:75) = 26			Number of take-offs per mosquito (705:75) = 9.4		

TABLE 2. IRRITABILITY TEST

Batches of five mosquitos - Anopheles atroparvus, Italian strain
Conical exposure chambers. Incident light 50 Lux. Temperature 20°-25°C. Relative humidity 58-60%

Females engorged at indicated time before testing

Time after last blood meal	Serial Number	DDT 4% Number of take-offs in 15'		Serial Number	Control Number of take-offs in 15'	
		Total	Individual		Total	Individual
5'	1	40	8	1a	20	4
5'	2	15	3	2a	5	1
30'	3	35	7	3a	10	2
60'	4	44	9	4a	19	4
60'	5	36	7	5a	16	3
60'	6	22	4	6a	25	5
60'	7	34	7	7a	14	3
60'	8	39	8	8a	5	1
65'	9	40	8	9a	25	5
75'	10	42	8	10a	15	8
120'	11	60	12	11a	60	12
120'	12	10	2	12a	10	2
180'	13	40	8	13a	10	2
180'	14	45	9	14a	12	2
240'	15	125	25	15a	15	3
Number of take-offs per mosquito (627:75) = 8.3				Number of take-offs per mosquito (261:75) = 3.3		

TABLE 3. IRRITABILITY TEST

Batches of five mosquitos - Anopheles labranchiae (Calalafimi, Trapani)
Conical exposure chambers. Incident light 50 Lux. Temperature 20°-25°C. Relative humidity 58-60%

Females engorged at unknown time before testing

Serial Number	DDT 2% Number of take-offs in 15'		Serial Number	DDT 4% Number of take-offs in 15'		Serial Number	Control Number of take-offs in 15'	
	a Total	b Individual		a Total	b Individual		a Total	b Individual
1	123	25	1a	90	18	1b	50	10
2	54	11	2a	35	7	2b	20	4
3	95	19	3a	110	25	3b	50	10
4	48	10	4a	40	8	4b	30	6
5	120	24	5a	90	18	5b	60	12
6	35	7	6a	60	12	6b	50	10
7	45	9	7a	85	17	7b	40	8
8	70	14	8a	65	18	8b	60	12
9	35	7	9a	80	16	9b	60	12
10	60	12	10a	71	14	10b	53	11
11	75	15	11a	105	24	11b	40	8
12	70	14	12a	92	18	12b	70	14
13	75	15	13a	75	13	13b	50	10
14	85	17	14a	80	16	14b	65	13
15	55	11	15a	35	7	15b	55	11
Number of take-offs per mosquito (1045:75) = 14			Number of take-offs per mosquito (1103:75) = 14.5			Number of take-offs per mosquito (753:75) = 10		

We have found that the intensity of light most favourable for reducing the number of movements to a minimum is 50 lux. A temperature of from 20° to 25°C and a relative humidity of from 55-60% also seem to reduce to a minimum the natural mobility of the mosquitos.

Tables 1, 2 and 3 show the results of these experiments.

An examination of the data presented in the tables leads to the following conclusions:

(a) the individuals of A. atroparvus in the hour following a blood meal, whether on treated or untreated surfaces, move much less than those fasted or those with a blood meal in an advanced state of digestion;

(b) the individuals of A. atroparvus exposed to treated surfaces move three times as much as do the controls (untreated surfaces);

(c) the concentration of DDT used does not cause any notable difference in the stimulus to take-off;

(d) the individuals of A. labranchiae exposed to treated surfaces seem to behave in a manner not significantly different from the controls.

These results, which correspond to those described by Zulueta (1958),[9] indicate that A. labranchiae is less irritable than A. atroparvus, and even the latter is only slightly irritable in comparison with other species.

3. Study of the susceptibility to insecticides of A. labranchiae in Sicily, comparing the WHO and Busvine-Nash methods

A comparative evaluation of the WHO and Busvine-Nash methods was done on about 15 000 A. labranchiae females.

When the Busvine-Nash method[1] is used on A. labranchiae, the average mortality is constantly higher than obtained with the WHO method.[7,8]

This result is contrary to those of various authors who have obtained equal results with both methods in other species of Anopheles.

In this laboratory also, no difference between the two methods has been found when working with laboratory-raised A. atroparvus. However, keeping in mind the results of the irritability tests reported above, it seems likely that the difference in mortality obtained using the two methods on A. labranchiae are due to the fact that the individuals of this species are less irritable and tend to stay in the position assumed at the moment of their introduction into the tubes. It occurs that several individuals remain on the metal screen (untreated) which closes the tubes, without being disturbed by the others flying, thus avoiding contact with the treated surface.

Table 4 shows the comparative data obtained in August 1959, using both methods. The differences are slight, but constant. The tests were carried out simultaneously in order to avoid the effects of the seasonal changes in sensitivity.

4. Study of resistance and susceptibility of Anopheles to organophosphorus compounds

(a) Experiments to study the residual action of some phosphorus esters on A. atroparvus and A. labranchiae

The residual insecticidal action has been studied of wall surfaces treated with phosphorus esters alone or associated with chlorinated insecticides.

In order to avoid the influence of previous treatments, the walls of the dwellings chosen for these experiments were first scraped and then repainted with water-base paints.

On 11 November 1959, a single wall in each of the five rooms was sprayed by means of pressure pumps with the following preparations and intended dosages (in terms of the technical product or of the pure active product):

(1) 30% malathion, technical 20% emulsifiers; 50% Kaolin, applied in a 2% water emulsion, at 0.4 g/m^2;

(2) 50% malathion, technical 50% solvents and emulsifiers, applied in a 2% water emulsion, at 0.66 g/m^2;

TABLE 4. DDT SUSCEPTIBILITY OF A. LABRANCHIAE ACCORDING TO THE WHO AND BUSVINE & NASH METHODS

District and locality	WHO method					Busvine/Nash method				
	Number of mosquitos	DDT concentration in risella oil				Number of mosquitos	DDT concentration in risella oil			
		0.5%	1%	2%	4%		0.5%	1%	2%	4%
Palermo (treated area)	3 862	14.3	43.7	74.3	100	500	14.0	32.0	77.0	100
Palermo (untreated area)	860	8.0	49.5	90.0	100	480	17.0	55.0	96.5	100
Catania	2 025	50.0	58.3	83.2	100	645	39.0	53.3	85.5	100
Trapani	2 045	41.6	46.5	76.6	100	192	48.0	62.0	83.5	100
Agrigento	1 033	39.0	73.0	87.3	100	150	36.0	66.0	89.0	100
Caltanissetta	1 660	27.0	59.9	87.9	100	525	-	67.0	75.0	100
Siracusa	760	40.0	58.0	83.0	100	560	60.0	72.0	90.0	100
Totals	12 252	31.4	55.5	83.1	100	3 052	35.6	58.1	85.3	100

Tests carried out in August 1959

TABLE 5. RESIDUAL EFFECT OF WALLS TREATED WITH ORGANOPHOSPHORUS COMPOUNDS ON 10 NOVEMBER 1959

	Insecticide	Per cent. mortality[a] of *A. atroparvus* after 15-minute exposure										Per cent. mortality of *A. labranchiae* after 15-minute exposure		
		Months after treatment										Months after treatment		
		1	2	3	4	5	6	7	8	8[b]	10[c]	8	9	10[c]
1.	Malathion paste	100	100	90	90	65	65	65	60	70	20	80	50	35
2.	Malathion liquid	100	90	80	70	35	35	35	65	30	20	80	40	30
3.	Malathion synergized	100	100	90	90	52	50	50	40	80	50	100	95	65
4.	Dithion 10 Pb.	100	100	80	80	30	30	30	25	50	60	90	90	80
5.	Dithion polyvalent paste	100	100	100	80	80	70	70	70	80	40	100	100	55

[a] Corrected by Abbott's formula

[b] Test carried out 8 months after treatment but with 30' exposure

[c] Test simultaneously carried out on *atroparvus* and *labranchiae*

(3) 50% malathion, technical; 14% lindane; 36% solvents and emulsifiers, applied in a 2% water emulsion, at 0.85 g/m^2 (malathion 0.665; lindane 0.185);

(4) 10% dithion (o.o diethylthiophosphate of 7-oxy-3-tetramethylencoumarin); 5% Rogor (N-methylamide of dimethyl-dithiophosphoryl acetic acid); 85% auxiliary and inert ingredients: applied in a 6.66% suspension, at 0.61 g/m^2 (dithion 0.41; Rogor 0.2);

(5) 18% dithion; 5% Rogor; 54.5% DDT; 22.5% diluents and dispersants: applied in a 6.66% water emulsion, at 3.22 g/m^2 (dithion 0.75; Rogor 0.2; DDT 2.27).

One litre of each formulation has been sprayed over 15 m^2 of wall surface (= 66 ml/m^2). The insecticidal action of these walls tested by means of the conical exposure chambers (WHO, Technical Report Series, No. 153, Geneva, 1958, p. 8) with laboratory-raised females of *A. atroparvus*. Some tests were also done with *A. labranchiae*.

A preliminary test had been made to make certain that the walls had no insecticidal action before treatment.

After the treatment, the tests were carried out every 15 days, first with laboratory-raised *A. atroparvus* and later with *A. labranchiae* which had been captured in their natural environment.

Since an exposure of 30 minutes (as recommended in the above-mentioned Technical Report) produced 100% mortality of mosquitos, the time of exposure was reduced to 15 minutes and kept as such during the whole course of the experiment. In spite of this, in the first months after treatment the mortality remained at 100% for all the insecticides used.

The results of these tests are shown in Table 5. In this table each mortality figure is the average of three tests: in each test, 10 females have been used, with 10 females exposed to an untreated wall of the same room, acting as controls.

A limited number of irritability tests were also done on the walls treated with the above-mentioned insecticides. The tests were made according to variation (b) of the WHO method, using *A. atroparvus* and *A. labranchiae*. These tests have demonstrated that these species show only slight irritability towards the insecticides used.

The above experiments make evident the following facts:

(a) The physical properties of the insecticide have an obvious influence on the duration of the action. This is particularly noticeable in a comparison between insecticides No. 1 and No. 2. In fact, insecticide 1, although it contained only 30% of the active ingredient (malathion), had a longer duration of action than No. 2 which contained 50% of the same substance.

(b) It is obvious that the residual action of the organophosphorus insecticides on the species atroparvus and labranchiae is notable enough to be of practical importance. In fact, taking into consideration that after a blood meal the mosquitos remain on the walls of a dwelling for quite some time, and certainly always more than 15 minutes, the results of these experiments indicate that one-yearly treatment with the insecticides described would be sufficient.

A consistently higher mortality of A. labranchiae, compared with A. atroparvus, has been noticed. Though it is probable that a more pronounced excitability of atroparvus may play a role, data are not available on the irritability of A. labranchiae to organophosphorus insecticides to warrant conclusion.

A certain degree of rigour tolerance in the strain of A. atroparvus tested appears to be evident. However, the matter is still awaiting data and is thus open to discussion.

(b) Research on a procedure to determine the degree of susceptibility of Anopheles to organophosphorus esters

We have followed as closely as possible the technical standards described by the WHO for testing the susceptibility to chlorinated insecticides.[8]

The following variations were introduced:

1. Since the plastic tubes are attacked by the phosphorus-esters insecticides, they were replaced by glass tubes, which were also more easily washed. The open end of the tube was closed with a cotton netting held in place by an elastic band, instead of the metal screen. Those tubes which contained insecticide-impregnated paper were closed with netting which had also been impregnated by a previous immersion in the insecticide solution.

2. During the period of exposure of the mosquito (one hour), the tubes were kept in a horizontal rather than in a vertical position. In fact, in the horizontal tubes the anophelines are much less mobile, in contrast to the situation in the vertical tubes, where the mosquitos disturbed one another because of their tendency to make for the netting covering the opening.

3. The use of other solvents than risella oil and trichlorethylene which were found to be unsatisfactory for the tests with phosphorus esters.

4. Solutions of active substances were prepared at the final desired concentration in order to avoid further dilution.

The experiments were done only with malathion, which was obtained in a pure state. In the case of the phosphorus esters the purity of the insecticide is an indispensable condition since the commercial products are less stable and vary in chemical characteristics.

The search for the correct solvent. This is the crucial point in the establishment of a procedure to measure the susceptibility of the *Anopheles* to organophosphorus insecticides.

Unsatisfactory results were obtained with the following solvents: risella oil, benzene and butyl-stearate. The reasons for failure were the difficulty in obtaining reproducible results, especially with risella oil and butyl stearate, and, in case of benzene, also the brief duration of the activity of the impregnated paper.

At the moment we are considering experiments using olive oil as a solvent as suggested by Davidson; meanwhile, we present certain data on the use of a solution of colophony in acetone.

Tests using acetone solutions of Colophony Common Resin

It was presupposed that after the evaporation of the acetone the resin would hold the malathion on the treated walls, giving it off gradually and uniformly.

The colophony used was the pale yellow type which corresponds to the American standard referred to as "superior M". Various solutions of malathion were prepared in 20% colophony in acetone. The solutions were kept in glass-stoppered bottles at a low temperature.

TABLE 6. SUSCEPTIBILITY OF A. ATROPARVUS AND A. LABRANCHIAE TO MALATHION IN COLOPHONY
(malathion 0.5% in acetone + 20% colophony common resin)

Papers impregnated with 2 ml of the 1:3 dilution in acetone
and kept in plastic containers 3 days
(1-hour exposure. Temperature 26-27°C. Relative humidity 65-68%)

Numbers tested: 25 ♀ per test, 25 ♀ control

Papers impregnated on	Date of test	Per cent. corrected mortality			
		Anopheles atroparvus			A. labranchiae (Wild)
		Italian strain	Hamburg strain	Malathion strain	
17 October 1960	9 November		70		16
17 October 1960	12 November			47	
17 October 1960	15 November		80		32
17 October 1960	17 November	75		52	
17 October 1960	26 November	55			25
17 October 1960	2 December				28
17 October 1960	7 December		52		
17 October 1960	7 December		68		

TABLE 7. SUSCEPTIBILITY OF ANOPHELES ATROPARVUS TO MALATHION
(malathion 0.5% in acetone + 20% colophony common resin)

Papers impregnated with 2 ml of the 1:3 dilution in acetone
(1-hour exposure. Temperature 26-27°C. Relative humidity 65-68%)

Numbers tested: 25 ♀ per test, 25 ♀ control

Papers impregnated on	Date of test	Per cent. corrected mortality of *A. atroparvus*		
		Italian strain	Hamburg strain	Malathion strain
10 October 1960	11 October 1960		96	
10 October 1960	12 October 1960		100	
10 October 1960	15 October 1960		100	
10 October 1960	16 October 1960		72	
10 October 1960	18 October 1960		75	
10 October 1960	19 October 1960		70	
17 October 1960	18 October 1960		100	
17 October 1960	19 October 1960		100	
17 October 1960	20 October 1960		85	
17 October 1960	21 October 1960		96	
17 October 1960	21 October 1960			56
17 October 1960	22 October 1960		95	56
17 October 1960	25 October 1960		75	
17 October 1960	27 October 1960		70	
17 October 1960	1 November 1960		35	
17 October 1960	5 November 1960		35	
17 October 1960	26 November 1960	60		
17 October 1960	27 November 1960	72		
17 October 1960	30 November 1960		30	
17 October 1960	2 December 1960		44	
17 October 1960	7 December 1960		44	

TABLE 8. SUSCEPTIBILITY OF ANOPHELES ATROPARVUS AND ANOPHELES LABRANCHIAE TO MALATHION
(malathion 0.5% in acetone + 20% colophony common resin)

Papers impregnated with 2 ml of indicated solution without further dilution

Numbers tested: 25 ♀ per test, 25 ♀ control

Papers impregnated on	Date of test	Per cent. corrected mortality			
		Anopheles atroparvus			A. labranchiae (Wild)
		Italian strain	Hamburg strain	Malathion strain	
16 November 1960	19 November 1960		100		
16 November 1960	19 November 1960				33
16 November 1960	21 November 1960		62		
16 November 1960	24 November 1960	93			
16 November 1960	27 November 1960	80			
16 November 1960	30 November 1960		67		
16 November 1960	2 December 1960		72		
16 November 1960	7 December 1960		80		
16 November 1960	8 December 1960	68			
10 December 1960	14 December 1960		70		
10 December 1960	14 December 1960		70		
10 December 1960	16 December 1960		100		
10 December 1960	17 December 1960			50	
10 December 1960	18 December 1960	80			
10 December 1960	4 January 1961		96	12	
10 December 1960	11 January 1961		84		

The fluctuation in mortality observed with the Hamburg strain is not easy to explain. The 80% mortality obtained on 7 December 1960, using the same impregnated papers, would exclude the ageing of papers as a cause. Minor variations of temperature could offer an explanation since it was recently noticed that a rise in temperature of 1°C between 25°C and 27°C produced higher mortality. These factors are now being investigated.

Two groups of tests were performed: in the first group, the solutions of insecticide were diluted with two volumes of acetone before being used to impregnate the paper. In the second group the paper was impregnated directly with the solution in colophony, without further dilution. In both cases, rectangles of Whatman No. 1 paper were treated with 2 cc of the solution in the usual way. The pieces of netting for closing the tubes were immersed in the solution, drained and dried in the same way as the paper. In the intervals between one experiment and another the tubes containing paper impregnated with insecticide were kept in plastic bags.

The number of mosquitos in each test was 25 females. An equal number of females served as control. The mortality figures have been corrected with Abbott's formula. In general, mortality amongst control was nil.

The results are shown in Tables 6, 7 and 8.

Besides the tests carried out using 0.5% malathion, as shown in Tables 6, 7 and 8, we have also done some tests using concentrations of 0.25 and 1.0%. We have observed that a concentration of 0.25% produced a very low rate or nil mortality. The concentration of 1.0%, however, brought about 100% mortality in all the strains we have studied.

The above results are preliminary: they show that the addition of colophony to the acetone solution of malathion is a useful expedient. When this method has been perfected, it will perhaps be possible to establish an accurate procedure for the measurement of the susceptibility of anophelines to organophosphorus compounds.

The following observations were also made during the experiments using colophony:

(i) When the concentration of resin is increased, it is also necessary to increase the concentration of malathion in the same proportion: for instance, if the concentration of resin is doubled, the concentration of malathion should also be doubled.

(ii) With the solutions we have used up to now, the successive concentrations of malathion on a logarithmic scale do not give a gradual increase in mortality and, as a result, it has not been possible to establish a precise LC_{50}.

(iii) The appreciable duration of the activity of the papers used seems to be due to the thin film of resin which encloses the insecticide.

(c) The selection of a strain of A. atroparvus exposed to malathion

We are at present attempting to select a malathion-resistant strain of A. atroparvus.

For this purpose we have used the Anopheles which have survived various treatments with malathion. Each generation of larvae and adults are exposed to the action of the insecticide. The larvae are raised in water containing 0.035 p.p.m. of malathion and the adults are exposed to papers impregnated with a solution of malathion in benzene prepared four days before each experiment. For the selection of the strain we preferred to use the solution in benzene rather than that in acetone and colophony because the former facilitates the computation of the LC_{50}. In fact, the same papers have been used to measure the LC_{50} of the colony exposed to the pressure of malathion for six generations, as well as for the Italian and Hamburg strains of A. atroparvus.

The following table shows the mortality obtained with 500 individuals of each strain and the corresponding LC_{50}.

TABLE 9

Concentration of malathion in benzene	Per cent. mortality of A. atroparvus after 1-hour exposure (°)		
	Italian strain	Hamburg strain	Malathion strain
0.05%	37%	14.5%	0%
0.1%	96%	72%	21.7%
0.2%	100%	100%	65%
LC_{50}	0.058	0.072	0.18

(°) Mortality counts done after 24 hours, corrected with Abbott's formula

Altogether, 4500 female *atroparvus* have been employed, 350 per test with 150 as controls.

From the above data it is evident that the colony established by selection is actually only three times less susceptible than the original colony (Italian). The reduction in susceptibility is probably due only to vigour tolerance.

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