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Observations on the Irritability of Mosquitos to DDT in Uganda *

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The need for new investigations on the effect of insecticides on the behaviour of mosquitos was recently stressed by the WHO Expert Committee on Insecticides, which recommended a provisional method for the determination of the irritability of mosquitos to insecticides for trials in the field and the laboratory. One such trial, carried out in Uganda under what can be termed typical field conditions, is reported in the present paper.

Two strains of Anopheles gambiae, *one of* Anopheles funestus, *one of* Anopheles pharoensis *and one of* Aëdes aegypti *were tested for their irritability to DDT.* Anopheles gambiae *proved by far the most irritable of the species examined but in all the trials, the authors point out, there was a great individual variation even when the tests were carried out at the same time of the day and with mosquitos of the same age and condition of feeding. Flights in the control mosquitos were also noticeable and the provisional method was not considered entirely satisfactory for use in the field.*

A series of observations on the irritability of mosquitos to DDT is reported in this paper. Three populations from the north of Kigezi District, Uganda, as well as two laboratory strains maintained in our insectary, were examined. Two of the Kigezi populations—*Anopheles gambiae* and *Anopheles pharoensis* from the north of the District—had been subject to DDT pressure for over a year. The other material, as far as we can ascertain, had been free from insecticide pressure. In all cases normal levels of susceptibility were found when using the method for the determination of susceptibility in adult mosquitos recommended by the WHO Expert Committee on Insecticides (1960). The observations made afforded also a good opportunity to assess the value of the provisional method for the determination of irritability in mosquitos, recommended by the same Committee, under what can be described as typical field conditions.

Before the reader enters into the details of our work we should point out that the provisional method for irritability determination in its original form, or with the slight modifications introduced by us, did not prove entirely satisfactory under the simple conditions of our field laboratory where, as will be explained later, there was no control of humidity and temperature. Our efforts to provide adequate conditioning of the mosquitos (by keeping them before the test separately in individual paper cups) were not entirely satisfactory, nor were our endeavours to reduce to a minimum the activity due to the effect of light (there were always flights in the controls in all our trials). Altogether the provisional method, whether unchanged or with the slight modifications introduced by us, did not seem to be entirely adequate to ascertain the irritability of mosquitos under field conditions. In our view important modifications would be necessary before this method could be considered a useful tool for the entomologist. A. and M. Coluzzi have recently evolved [3] what appears to be a highly satisfactory method for the testing of irritability in mosquitos. Although it embodies some of the principles of the provisional method recommended by the WHO Expert Committee on Insecticides, it is in fact a new test method in which the problems of conditioning and illumination appear to be satisfactorily solved.

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[3] Coluzzi, M. (1961) *Essai d'élaboration d'une procédure expérimentale pour déterminer l'irritabilité des moustiques adultes aux insecticides* (unpublished report to WHO).

The new Coluzzi method will make it possible to determine the irritability of mosquitos in a much more accurate way than was possible before, but nevertheless some of the previous observations carried out with less sensitive methods will still be of use to the entomologist. This, we believe, is the case with the series of observations presented in this report. They confirm in the first place the very great irritability of *Anopheles gambiae* already noted by Hamon & Eyraud (1961), Mouchet et al. (1961), and more recently by Coluzzi (*op. cit.*) using his new method. They confirm also what is probably more important—the much higher irritability of *Anopheles gambiae* compared with *Anopheles funestus*. This had been noted by Hamon & Eyraud (1961) in the Upper Volta and by Mouchet et al. (1961) in the Cameroons. Our observations also confirm the remarkable fact, already noticed when determining the irritability of mosquitos, that 15 minutes' exposure to papers impregnated with 2% or 4% DDT (WHO papers for the determination of susceptibility) produce little or no mortality when the mosquitos are exposed singly to the treated papers.

Furthermore, our observations, which in one experiment were carried out through three consecutive generations of *Anopheles gambiae*, show the high degree of individual variation to be expected when determining the irritability of a given population. The more sensitive method of Coluzzi has incidentally confirmed this point.

METHODS

All the experiments reported in this paper were carried out in the laboratory at Kihihi, North Kigezi, Uganda. This laboratory, situated less than 1° south of the Equator, at 3700 feet (1130 m) above sea level, is built of mud and wattle and mosquito screening. No temperature or humidity control is possible, but light can be varied by the raising or lowering of blinds over the mosquito screening.

The instructions contained in Annex 6 of the report of the WHO Expert Committee on Insecticides (1960) were used as a basis for the experiments. Transparent conical exposure chambers (as used in the WHO bio-assay test) were mounted vertically over the exposure papers on a sheet of hardboard by means of adhesive plaster. The impregnated 4% DDT and 2% DDT papers and the non-impregnated control papers used for susceptibility testing, and contained in the WHO adult mosquito test kit, were used as exposure papers.

Mosquitos introduced into the chambers were exposed: (1) individually, and (2) in replicates of five at a time. In both cases 3 minutes were allowed as a " settling period " before the " number of take-offs " in the following 15 minutes was counted, using hand-tally counters. During the individual exposures, the " time of first take-off " (after the 3-minute settling period) was also recorded.

For each species of mosquito, 20 individual exposures, and 40 in replicates of five (8×5), were made at each concentration. For the " time of first take-off " no mosquito was watched for longer than 15 minutes, as experience showed that in 200 individual exposures at 4% and 2% DDT only 7 mosquitos did not fly during the 15-minute observation period (5 *Aëdes aegypti*, 1 *Anopheles gambiae* (North Kigezi) and 1 *Anopheles pharoensis*). Similarly with the 90 controls, 15 minutes was taken as the maximum, and where a mosquito did not fly, the " time of first take-off " was recorded as 15 minutes. Hence the control " time of first take-off " is in every case much lower than if the mosquitos had been observed for 90 minutes, for example.

The mosquitos used in the experiment were: (1) *Anopheles gambiae* (North Kigezi), (2) *Anopheles gambiae* (Kisumu strain—Kihihi colony), (3) *Anopheles pharoensis* (North Kigezi), (4) *Anopheles funestus* (South Kigezi) and (5) *Aëdes aegypti* (E.A.V.R.I. strain—Kihihi colony). With all these, except *Anopheles funestus*, laboratory-reared adults were used. *Anopheles gambiae* (Kisumu strain) and *Aëdes aegypti* (E.A.V.R.I. strain) were from our own colonies at Kihihi. *Anopheles gambiae* (North Kigezi) and *Anopheles pharoensis* (North Kigezi) were reared from eggs from wild-caught females. It proved difficult to rear *Anopheles funestus* in sufficient numbers in the laboratory, so wild-caught females were brought from Lake Bunyoni in South Kigezi; these were kept in the laboratory for three days, blood-fed, and exposed the following day. All the other species were blood-fed at two days old and exposed the following day, all being kept in darkness from emergence until feeding, and again from then until exposure. Human blood was used, and only well-gorged, undamaged females were tested.

All the exposures took place in daylight, adjusted to 40 foot-candles (measured by Weston Master III light-meter) by the raising or lowering of a blind in the laboratory. The temperature and humidity in the laboratory during the exposures were recorded. After exposure the mosquitos were removed from

the conical chambers and kept in unwaxed paper cups, with glucose solution on cotton-wool, for 24 hours, when the mortalities were counted.

RESULTS

Experiment No. 1

The results of this experiment are recorded in Table 1, which shows (*a*) the average number of take-offs per mosquito in 15 minutes (after 3-minute settling period) for individual exposures, (*b*) the average time of first take-off (after 3-minute settling period), and (*c*) the average number of take-offs per mosquito in 15 minutes (after 3-minute settling period) in replicates of 5 mosquitos at a time. The accompanying figure records these results pictorially.

The LC_{50} of the different species, where tested, is also given in Table 1, but there appears to be no relationship between this and the irritability of the mosquitos. The two *Anopheles gambiae* strains and the *Anopheles funestus* show a relationship of decreasing irritability with decreasing LC_{50}, but the trend is completely reversed by the *Aëdes aegypti*, which shows the lowest irritability with the highest LC_{50}.

Experiment No. 2

From the results of the first experiment it was thought that the variance within the species was very high, particularly with the more irritable mosquitos, i.e., *Anopheles gambiae* (North Kigezi) and (Kihihi colony), *Anopheles pharoensis*, and even *Anopheles funestus* at 4% DDT. It was decided then to carry out further experiments to see whether this variance could be reduced at all by controlling factors other than the DDT.

In the first instance it was thought that mosquitos which had been fed and then allowed to remain together in their cage along with the males might be more excitable than isolated females. In order to determine this, *Anopheles gambiae* of the Kihihi colony (approximately 50% females and 50% males) were allowed to emerge as usual into a 1-foot-cube cage ($30 \times 30 \times 30$ cm) and remain thus for 48 hours, when they were blood-fed; some were then removed to unwaxed paper cups, one blood-fed female per cup. All were kept in darkness in the laboratory until exposure the following day.

As 4% DDT had usually shown a higher variance than 2% DDT, it was decided to use the latter concentration only, and exposures were made to: (1) 2% DDT from the cage; (2) 2% DDT from individual cups; and (3) control papers from the cage. Individual exposures only were made. The results of this experiment are shown in Table 2, where it will be seen that the average number of take-offs from individual cups was considerably lower than that from the cage, and that the time of first take-off was correspondingly longer. However only the latter difference was significant ($P = < 1\%$).

Experiment No. 3

During the course of the second experiment it was thought that some difference in irritability could be discerned between exposures carried out in the mornings and those in the afternoons.

A further experiment was designed to test this, in which *Anopheles gambiae* (Kihihi colony) were blood-fed between 14.15 and 14.45 hours when the mosquitos were two days old. Well-gorged, undamaged females were then isolated, one per paper cup, and kept in darkness until the following day. Exposures were then made in the mornings between 09.30 and 13.00 hours, and in the afternoons between 15.00 and 17.30 hours. This experiment was extended over three generations of the colony (No. 7, 8 and 9), in the first of which only seven morning and six afternoon exposures were made, but in the two succeeding generations 28 mosquitos were tested in the morning and 24 in the afternoon. These exposures were of individual mosquitos only. In generation No. 7 only one replicate of 2% DDT and one control for one morning and afternoon were tested. In generations 8 and 9, two individual replicates of 2% DDT and one control on each of two successive mornings and afternoons were exposed.

The results of this experiment are given in Table 3, from which it will be seen that the mean number of take-offs per mosquito for 2% DDT is very close in both the morning and the afternoon (24.92 and 24.37). However, the average time of first take-off shows a difference (1.57 minutes and 3.88 minutes) which is significant ($P = < 0.1\%$). In the controls, this difference can be seen not only in the average time of first take-off but also in the average number of take-offs.

Experiment No. 4

A short experiment was carried out to observe the irritability of *Anopheles gambiae* (Kihihi colony) on repeated exposure to DDT.

As in the previous experiment the mosquitos were blood-fed between 14.15 and 14.45 hours when they

TABLE
MOSQUITO IRRITABILITY TO

Species	Concentration	Individual									
		No. of take-offs in 15 minutes (after 3-minute settling period)						Time of first take-off (after 3-minute settling period)			
		No. of mosquitos tested	Av. no. of take-offs per mosquito	SE [a]	Variance	Range in no. of take-offs per mosquito		No. of mosquitos tested	Av. time of first take-off (minutes)	SE [a]	Variance
						Min.	Max.				
Anopheles gambiae (North Kigezi)	4% DDT	20	25.5	3.10	193	6	59	20	2.1	0.48	4.6
	2% DDT	20	20.6	2.34	109	0	47	20	3.2	0.75	11.3
	Control	20	1.1	0.43	3.7	0	11	10	12.4	1.72	29.6
Anopheles gambiae (Kihihi colony)	4% DDT	20	21.8	3.28	215	2	60	20	2.8	0.52	5.3
	2% DDT	20	13.9	2.36	111	1	30	20	6.4	1.12	25.1
	Control	20	0.3	0.18	0.66	0	3	20	12.3	1.25	31.4
Anopheles pharoensis (North Kigezi)	4% DDT	20	16.2	3.04	185	1	44	20	4.2	0.93	17.3
	2% DDT	20	14.1	3.23	209	0	40	20	6.8	1.14	26.1
	Control	20	3.1	1.51	45.6	0	29	20	10.6	1.33	35.3
Anopheles funestus (South Kigezi)	4% DDT	21	8.8	1.73	65.3	1	30	20	4.2	0.69	9.8
	2% DDT	21	6.2	0.85	15.1	2	17	20	4.9	0.88	15.6
	Control	22	0.68	0.35	2.6	0	5	20	14.1	0.70	10.0
Aëdes aegypti (Kihihi colony)	4% DDT	20	2.6	0.52	5.4	0	9	20	2.4	0.97	18.1
	2% DDT	20	3.1	0.51	5.1	0	8	20	4.2	1.11	24.7
	Control	20	1.1	0.49	4.8	0	8	20	10.8	1.47	43.1

[a] SE = standard error.
[b] RH = relative humidity during test.

were two days old and isolated individually in numbered paper cups. Individual exposures to 2% DDT were carried out the following day (i.e., at approximately 24 hours after feeding) and the time of exposure for each mosquito was recorded. After the test each mosquito was replaced in its paper cup and kept in darkness for a further 24 hours, when they were all re-exposed to 2% DDT at approximately the same time of day (i.e., approximately 48 hours after feeding). They were kept a further 24 hours to observe the mortality.

It will be seen from Table 4 that there is a wide difference between the means of the first and second exposures, both in the average number of take-offs and in the average time of first take-off; both these differences are significant. It is interesting to note that in every case the number of take-offs was lower on the second than on the first exposure, and that only three times of first take-off were shorter on the repeated test. It is not clear from our records whether this is due to different ages or to different degrees of ovarian development.

Experiment No. 5

In order to investigate the effect of colour and oiliness of the exposure papers, a further experiment was carried out using: (1) a black control paper; (2) a plain white control paper; and (3) a standard WHO Risella oil control paper. The black control was made by soaking a Whatman No. 1 filter paper in black Indian ink and allowing it to dry. The plain white control was a Whatman No. 1 filter paper.

1

DDT (EXPERIMENT No. 1)

exposures					Exposures in replicates of 5									
					No. of take-offs in 15 minutes (after 3-minute settling period)									
Range in time of first take-off		24-hour mortality (%)	RH [b] (%)	Temp. during test (°C)	No. of mosquitos tested	Av. no. of take-offs per mosquito	SE [a]	Variance	Range in no. of take-offs per mosquito		24-hour mortality (%)	RH [b] (%)	Temp. during test (°C)	DDT LC_{50}
Min.	Max.								Min.	Max.				
0.01	6.75	17	54	21.6	40	39.8	2.29	41.8	29.0	46.8	73	54	21.6	
0.01	15.00	0	to	to	40	29.9	3.74	112	14.6	46.2	31	to	to	0.75
1.75	15.00	0	81	28.4	40	4.9	1.49	17.9	0.0	12.8	0	81	28.4	
0.03	9.03	11	54	21.6	40	38.5	5.59	250	27.2	43.2	70	54	21.6	
0.03	14.31	0	to	to	40	25.1	2.64	55.9	13.2	36.0	6	to	to	0.62
0.48	15.00	0	81	28.4	40	1.3	0.55	2.5	0.0	4.8	3	81	28.4	
0.02	12.11	15	65	21.3	25	35.8	2.82	39.9	28.2	45.0	25	65	21.3	Not tested
0.07	15.00	11	to	to	25	36.2	2.40	28.9	30.6	43.0	12	to	to	
0.07	15.00	0	76	24.4	20	3.7	3.16	39.9	0.0	13.2	0	76	24.4	
0.30	12.12	36	54	27.6	40	18.8	1.63	21.3	12.8	26.8	82	54	27.6	
0.72	13.88	24	to	to	40	17.4	1.76	24.9	6.8	21.4	35	to	to	0.35
1.08	15.00	5	63	28.2	40	11.4	0.69	3.8	8.4	14.0	0	63	28.2	
0.03	15.00	10	60	25.9	40	5.5	0.43	1.5	3.6	7.6	16	44	23.9	
0.13	15.00	5	to	to	40	6.8	1.26	12.6	3.0	11.4	2	to	to	2.5
0.50	15.00	0	61	26.9	40	3.1	0.95	7.2	1.2	8.0	0	47	28.5	

Anopheles gambiae (Kihihi colony) were blood-fed when two days old between 14.15 and 14.45 hours, isolated in paper cups and individually exposed the following day. The results were recorded for exposures made during the mornings between 09.30 and 13.00 hours, and during the afternoons between 15.00 and 17.30 hours.

Table 5 gives the morning and afternoon results of these tests, from which it will be seen that: (*a*) there is a considerable difference between the morning and afternoon in both the average number of take-offs and the average time of first take-off; and (*b*) the morning black control gave better results than either of the other two. (For the average number of take-offs, $P = < 5\%$, and for the average time of first take-off, $P = < 1\%$.)

Experiment No. 6

Continuing the investigation of exposure surfaces, another test was devised using mud-blocks. Two mud-blocks (in wooden frames, open back and front) 1 foot × 1 foot × 3 inches deep (30 × 30 × 7.5 cm) were made and allowed to dry out thoroughly. One was then sprayed with DDT wettable powder, at the rate of approximately 2 g/m². No special equipment was available for this, and a Hudson X-pert sprayer, as used by the spray teams in the Uganda Malaria Eradication Pilot Project, was used. The other block remained unsprayed as a control.

Anopheles gambiae (Kihihi colony), blood-fed at two days old, were exposed on the following day. The time of first take-off was recorded during the

MOSQUITO IRRITABILITY TO DDT (EXPERIMENT No. 1)

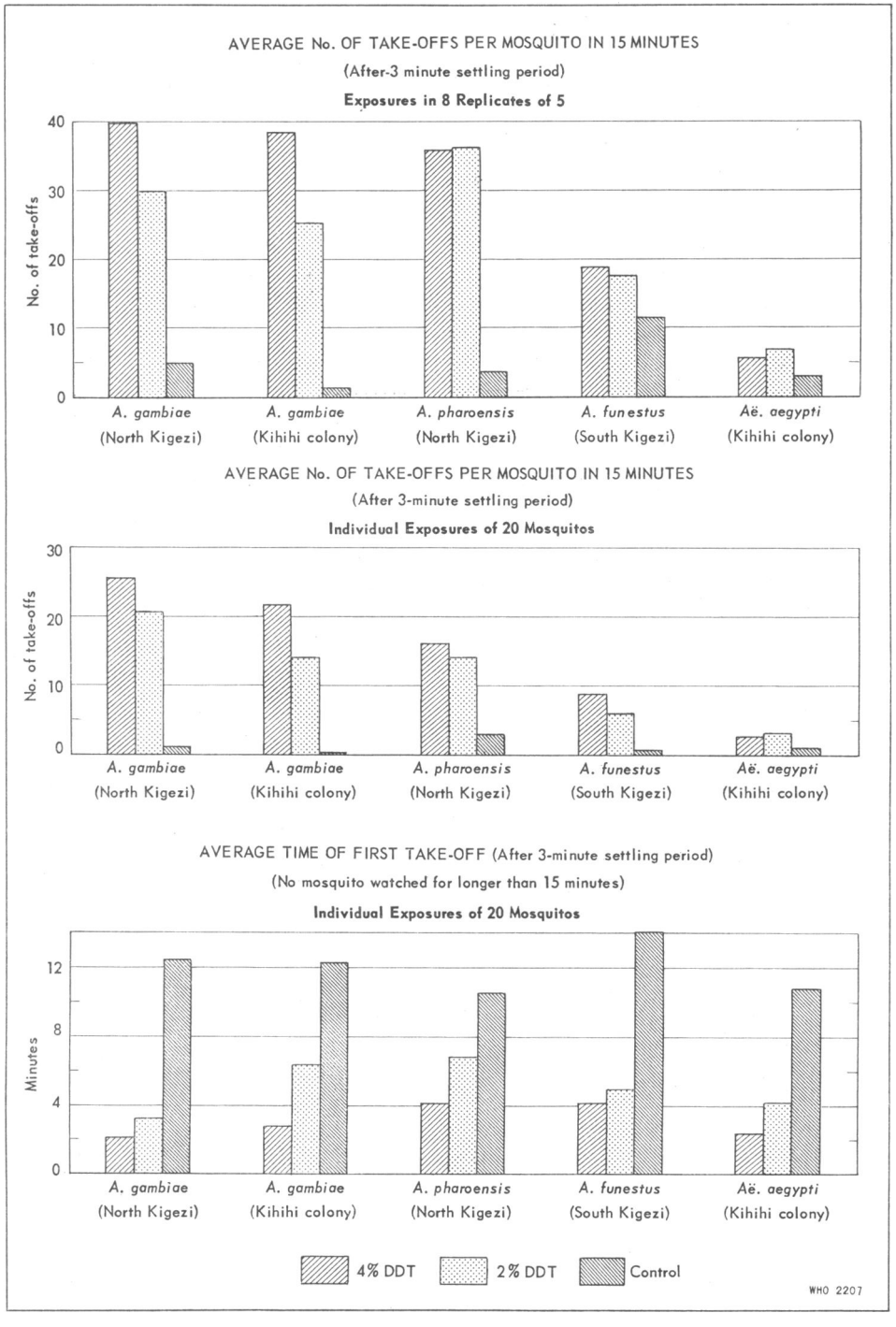

TABLE 2

COMPARISON OF IRRITABILITY BETWEEN "ISOLATED" AND "CAGED" *ANOPHELES GAMBIAE* (KIHIHI COLONY) IN INDIVIDUAL EXPOSURES (EXPERIMENT No. 2)

Resting state after feeding	Concentration	No. of take-offs in 15 minutes (after 3-minute settling period)						Time of first take-off (after 3-minute settling period)						24-hour mortality (%)	RH [b] (%)	Temp. during test (°C)
		No. of mosquitos tested	Av. no. of take-offs per mosquito	SE [a]	Variance	Range in no. of take-offs per mosquito		No. of mosquitos tested	Av. time of first take-off (minutes)	SE [a]	Variance	Range in time of first take-off				
						Min.	Max.					Min.	Max.			
Isolated	2% DDT	26	31.8	3.55	328	5	67	26	3.2	0.47	5.7	0.05	8.63	0	47	23.4
Caged	2% DDT	26	39.1	4.65	563	6	89	26	1.3	0.29	2.2	0.03	5.05	4	to	to
Caged	Control	26	1.3	0.45	5.3	0	9	26	10.4	1.20	37.6	0.43	15.00	0	69	28.6

[a] SE = standard error.
[b] RH = relative humidity during test.

individual exposures, as only one mosquito on the DDT mud-block did not fly in the 15-minute test period. A test using replicates of 5 mosquitos at a time was also carried out.

The results in Table 6 show that, in the mud-block experiments, the irritability, as reflected by the average number of take-offs, was very much less than in the preceding individual 2% DDT test. The average time of first take-off is also greater than in all the foregoing tests except the first. Exposures in replicates of 5 gave results very similar to those of the original experiment.

The control would tend to show that the mud-block is a more satisfactory surface than filter paper for this test, as during the individual exposures, of which 14 were in the morning and six in the afternoon, only two mosquitos flew during the 15-minute test period (both in the morning). The control exposures by replicates of 5, which were equally divided between the morning and afternoon, showed no movement whatsoever during the test period.

The above findings are of considerable interest. They show that DDT crystals on a mud surface produce less irritability and higher mortality than the DDT in the Risella oil solution of the WHO papers. It should be noted, however, that a strict comparison cannot be made in this case since there is no correspondence of dosage between 2% papers and 2 g/m².

Experiment No. 7

In order to determine the effect of reduced light on the irritability of *Anopheles gambiae* (Kihihi colony), exposures to 2% DDT and control papers were made at night. The lens of a Hunter 8-cell electric torch was masked with white filter paper until only the smallest amount of light necessary to observe the mosquitos was obtained. No reading on the Weston Master III light-meter could be detected at the exposure surface. The torch was placed on the bench between the observers and the apparatus, with the lens pointed downwards, so that only reflected light illuminated the exposure chambers. All curtains were drawn in the laboratory to exclude any outside light.

The mosquitos were blood-fed between 19.45 and 20.15 hours on the second day after emergence and were isolated individually in paper cups. Exposure took place the following night between 20.30 and 23.00 hours.

Minimum light would not appear to reduce the irritability of *Anopheles gambiae*. The results given in Table 7 show no diminution in the average number of take-offs, and an average time of first take-off half-way between the times obtained in the morning and afternoon in Experiment No. 3. Indeed, the control gives an increased average number of take-offs and a shorter average time of first take-off than any other control in the series of experiments.

DISCUSSION

It would appear that there is a wide variation of irritability to DDT between individual *Anopheles gambiae*. This was found in both the North Kigezi and the Kihihi colony (Kisumu strain) specimens.

TABLE 3

COMPARISON OF MORNING AND AFTERNOON IRRITABILITY TESTS OVER THREE GENERATIONS OF *ANOPHELES GAMBIAE* (KIHIHI COLONY) IN INDIVIDUAL EXPOSURES (EXPERIMENT No. 3)

Colony generation number	Concentration	No. of take-offs in 15 minutes (after 3-minute settling period)						Time of first take-off (after 3-minute settling period)						24-hour mortality (%)	RH [b] (%)	Temp. during test (°C)
		No. of mosquitos tested	Av. no. of take-offs per mosquito	SE [a]	Variance	Range in no. of take-offs per mosquito		No. of mosquitos tested	Av. time of first take-off (minutes)	SE [a]	Variance	Range in time of first take-off				
						Min.	Max.					Min.	Max.			
MORNING																
7	2% DDT	7	40.0	7.40	384	15	58	7	2.2	0.82	4.7	0.05	5.10	0	64	23.8
	Control	7	0.4	0.29	0.6	0	2	7	12.1	2.06	29.6	1.03	15.00	0	68	27.4
8	2% DDT	28	22.8	2.72	207	7	74	28	1.5	0.25	1.7	0.03	5.05	0	46	23.9
	Control	14	3.6	1.28	23.1	0	16	14	7.9	1.70	40.6	0.07	15.00	0	55	28.1
9	2% DDT	28	23.2	2.39	161	7	60	28	1.5	0.24	1.6	0.03	6.16	7	52	22.9
	Control	14	3.0	1.31	24.0	0	18	14	8.4	1.76	43.6	0.83	15.00	0	71	27.8
Morning total	2% DDT	63	24.9	1.89	226	7	74	63	1.6	0.18	1.9	0.03	6.16	3	46	22.9
	Control	35	2.7	0.75	19.6	0	18	35	8.9	0.83	41.9	0.07	15.00	0	71	28.1
AFTERNOON																
7	2% DDT	6	33.5	9.25	514	10	71	6	3.3	1.21	8.7	0.58	8.63	0	51	28.4
	Control	6	0.0	0.0	0.0	0	0	6	15.0	0.0	0.0	15.00	15.00	0	51	28.0
8	2% DDT	24	16.0	3.31	264	3	85	24	4.6	0.45	4.9	0.40	8.58	0	42	24.6
	Control	12	0.33	0.24	0.7	0	3	12	14.4	0.37	1.7	11.12	15.00	0	57	28.2
9	2% DDT	24	30.4	3.84	355	5	59	24	3.4	0.34	2.8	0.62	6.31	0	43	26.4
	Control	12	0.2	0.17	0.3	0	2	12	14.8	0.21	0.6	12.38	15.00	0	59	28.5
Afternoon total	2% DDT	54	24.37	2.70	394	3	85	54	3.9	0.29	4.5	0.40	8.63	0	42	24.6
	Control	30	0.2	0.12	0.44	0	3	30	14.7	0.17	0.9	11.12	15.00	0	59	28.5

[a] SE = standard error.
[b] RH = relative humidity during test.

This high individual variation was also noticeable in *Anopheles pharoensis*, and to a lesser extent in *Anopheles funestus* when exposed to the higher concentration of insecticide, and may be true of any species of mosquito which exhibits a high degree of irritability to DDT.

Efforts to reduce this variation, using the standard test equipment, were largely unsuccessful, though three factors of considerable interest were noted. The first of these is the very striking difference found, in Experiment No. 4, in the irritability of *Anopheles gambiae* (Kihihi colony) on repeated exposure. The decreased average number of take-offs and the longer average time of first take-off of the second exposure are significantly different from those of the first ($P = < 0.1\%$ in both cases). This is further emphasized by the fact that all the repeated exposures, without exception, gave a lower number of take-offs, and only three of them showed a reduced time of first take-off.

TABLE 4

IRRITABILITY OF *ANOPHELES GAMBIAE* (KIHIHI COLONY) TO DDT ON ORIGINAL AND REPEATED INDIVIDUAL EXPOSURES (EXPERIMENT No. 4)

Order of exposure	Concentration	No. of take-offs in 15 minutes (after 3-minute settling period)						Time of first take-off (after 3-minute settling period)						24-hour mortality (%)	RH [b] (%)	Temp. during test (°C)
		No. of mosquitos tested	Av. no. of take-offs per mosquito	SE [a]	Variance	Range in no. of take-offs per mosquito		No. of mosquitos tested	Av. time of first take-off (minutes)	SE [a]	Variance	Range in time of first take-off				
						Min.	Max.					Min.	Max.			
1st exposure (24 hours after blood meal)	2% DDT	38	43.7	3.97	600	10	115	38	1.18	0.29	3.3	0.01	8.07	0	49 to 56	28.7 to 29.4
2nd exposure (48 hours after blood meal)	2% DDT	38	12.8	2.42	222	1	65	38	4.98	0.48	8.8	1.08	13.66	0		

[a] SE = standard error.
[b] RH = relative humidity during test.

The second feature of interest was that exhibited in Experiments No. 5 and 6, in which a decreased irritation of *Anopheles gambiae* (Kihihi colony) was observed on (*a*) black control paper, and (*b*) mud-blocks. The black control gave morning results significantly different from the Risella oil control (and hence white control) in the average number of take-offs (P = < 5%) and in the average time

TABLE 5

IRRITABILITY OF *ANOPHELES GAMBIAE* (KIHIHI COLONY) IN INDIVIDUAL EXPOSURES ON BLACK, WHITE AND RISELLA-OIL CONTROL PAPERS (EXPERIMENT No. 5)

Exposure paper	No. of take-offs in 15 minutes (after 3-minute settling period)						Time of first take-off (after 3-minute settling period)						24-hour mortality (%)	RH [b] (%)	Temp. during test (°C)
	No. of mosquitos tested	Av. no. of take-offs per mosquito	SE [a]	Variance	Range in no. of take-offs per mosquito		No. of mosquitos tested	Av. time of first take-off (minutes)	SE [a]	Variance	Range in time of first take-off				
					Min.	Max.					Min.	Max.			
MORNING															
Black control	48	0.58	0.33	5.4	0	15	48	12.5	0.71	24.6	0.27	15.00	0	46 to 77	21.5 to 28.6
White control	48	2.37	0.59	16.9	0	21	48	8.0	1.17	65.9	0.58	15.00	0		
Risella-oil control	48	1.64	0.40	7.8	0	15	48	8.8	0.87	36.8	0.13	15.00	0		
AFTERNOON															
Black control	30	0.00	0.00	0.0	0	0	30	15.0	0.0	0.0	15.00	15.00	0	46 to 72	23.4 to 29.4
White control	30	0.30	0.59	1.0	0	4	30	14.6	0.32	3.1	5.55	15.00	3		
Risella-oil control	30	0.27	0.84	2.1	0	8	30	14.5	0.48	7.1	0.40	15.00	3		

[a] SE = standard error.
[b] RH = relative humidity during test.

TABLE

IRRITABILITY OF *ANOPHELES GAMBIAE* (KIHIHI COLONY)

Test surface and concentration	Individual									
	No. of take-offs in 15 minutes (after 3-minute settling period)						Time of first take-off (after 3-minute settling period)			
	No. of mosquitos tested	Av. no. of take-offs per mosquito	SE [a]	Variance	Range in no. of take-offs per mosquito		No. of mosquitos tested	Av. time of first take-off (minutes)	SE [a]	Variance
					Min.	Max.				
Mud-block, DDT at 2 g/m²	20	7.3	1.38	38.2	0	24	20	6.2	0.83	13.8
Mud-block, control	20	0.2	0.11	0.2	0	2	20	14.3	0.65	8.4

[a] SE = standard error.
[b] RH = relative humidity during test.

of first take-off (P = < 1%). The mud-blocks of Experiment No. 6 showed a very low level of irritability for individual exposures. The DDT mud-block gave an average number of take-offs lower than in any other experiment, indeed lower even than that for the repeated exposure in Experiment No. 4. The average time of first take-off was also seen to be considerably longer than any other, except that of 2% DDT in the original test. The control irritability was also widely different from that of any other test, lower even than the morning black control of Experiment No. 5. This would tend to suggest that it was the mud-block rather than the DDT formulation (wettable powder) that affected the level of irritability.

The third factor noted was the difference exhibited in irritability by *Anopheles gambiae* (Kihihi colony) between morning and afternoon exposures. This concerned more the onset of irritation in that it was the average time of first take-off which showed a considerable difference in Experiment No. 3, the longer afternoon average time of first take-off being significantly different from that of the morning (P = < 0.1%). The controls of this experiment, and those of Experiment No. 5, confirm this difference between morning and afternoon tests, not only in the average time of first take-off but also in the average number of take-offs. In both Experiment No. 3 and Experiment No. 5 the mosquitos were fed between 14.15 and 14.45 hours the day before exposure, and were tested during similar morning and afternoon periods. It is possible, therefore, that this " time of day " difference observed is related to the time elapsed since feeding. Similarly, it may be an effect of climatic differences. In both experiments the morning relative humidity was higher than that of the afternoon—though the relative humidity for

TABLE 7

IRRITABILITY OF *ANOPHELES GAMBIAE* (KIHIHI COLONY) TO DDT IN INDIVIDUAL EXPOSURES IN MINIMUM LIGHT

Concentration	No. of take-offs in 15 minutes (after 3-minute settling period)						Time of first take-off (after 3-minute settling period)						24-hour mortality (%)	RH [b] (%)	Temp. during test (°C)
	No. of mosquitos tested	Av. no. of take-offs per mosquito	SE [a]	Variance	Range in no. of take-offs per mosquito		No. of mosquitos tested	Av. time of first take-off (minutes)	SE [a]	Variance	Range in time of first take-off				
					Min.	Max.					Min.	Max.			
2% DDT	20	35.6	4.26	363	11	88	20	2.4	0.57	6.5	0.08	10.20	0	74 to 73	22.1 to 21.8
Control	20	5.9	1.54	47.8	0	21	20	7.0	1.52	46.7	0.17	15.00	0		

[a] SE = standard error.
[b] RH = relative humidity during test.

6

TO DDT ON MUD-BLOCK (EXPERIMENT No. 6)

exposures					Exposures in replicates of 5								
Range in time of first take-off		24-hour mortality (%)	RH [b] (%)	Temp. during test (°C)	No. of take-offs in 15 minutes (after 3-minute settling period)						24-hour mortality (%)	RH [b] (%)	Temp. during test (°C)
					No. of mosquitos tested	Av. no. of take-offs per mosquito	SE [a]	Variance	Range in no. of take-offs per mosquito				
Min.	Max.								Min.	Max.			
1.95	15.00	10	51 to	21.8 to	40	32.2	3.61	104	22.4	53.6	21	52 to	22.7 to
2.08	15.00	0	79	28.8	40	0.0	0.00	0.0	0.0	0.0	5	73	28.0

the morning in Experiment No. 3 (46%-71%) is almost identical with that in the afternoon for Experiment No. 5 (46%-72%)—and in both experiments the mean morning temperature is lower than that of the afternoon.

The combination of these three points raises interesting matter for speculation as to the effect of DDT on *Anopheles gambiae* in nature. A large part of the DDT-sprayed surfaces in this malaria eradication project, for example, are mud surfaces sprayed with wettable powder, which would appear to offer less irritation than that assumed from tests carried out with the WHO Risella-oil/DDT papers. If, also, this irritability is decreased by repeated exposure, and by " time of day " (possibly time elapsed since feeding, or climatic differences, or both), a level of irritability can be envisaged which would be low enough not to inhibit the lethal effect of the insecticide. All this underlines the need not to rely exclusively on irritability testing when trying to gauge the irritant effect of DDT in nature. Experimental huts fitted with window-traps can provide very useful additional information.

Mosquitos isolated in individual paper cups immediately after feeding showed a decrease in irritability compared with those remaining in the cage. Only the average time of first take-off, however, showed a significant difference of the means ($P = < 1\%$). Although the variance of the average number of take-offs was less from the isolated mosquitos, it was still very high, and the means were not significantly different.

Reducing the light to a minimum, as recommended by Sacca & Guy (1960) in a recent article, did not have the anticipated effect. In fact, the irritability of the control exposures was increased rather than decreased, the average number of take-offs being greater, and the average time of first take-off less, than in any other *Anopheles gambiae* (Kihihi colony) control. The 2% DDT exposure showed little change from the other experiments. Despite the narrow range of relative humidity and temperature during the test and the minimum light, the variance was still very great.

It would appear that the test method recommended is capable of showing variations of irritability between different species of mosquito, as was shown in the first test, and particularly such a broad difference as is exhibited between *Anopheles gambiae* and *Anopheles funestus*, for example. This difference appears best demonstrated by the average number of take-offs, with individual exposures, for this would seem to be less affected by " time of day " and other variations than is the average time of first take-off. Nor is it affected by a " proximity irritability " in the exposures by replicates of 5, as would appear to be the case of the *A. funestus* control (11.4 average take-offs per mosquito). However, 20 exposures as a sample would be too small if this alone were adopted, or even if the average time of first take-off were incorporated with it, as has been done in the present series of experiments.

CONCLUSIONS

The results of the work presented in this paper indicate that the provisional method for determining the irritability of adult mosquitos recommended by the WHO Expert Committee on Insecticides (1960) is not entirely satisfactory under conditions such as

exist in a field laboratory like our laboratory at Kihihi, particularly when dealing with a highly irritable strain. Under such conditions there is always activity due to factors other than the irritability produced by the DDT; for instance, the degree of illumination and the time of the day. The use of black papers may improve the test method but the replacement of standard WHO papers will meet with many difficulties. Mud-blocks provide a much better surface than impregnated papers but it does not seem possible to standardize mud-blocks and their insecticide dosage under field conditions. Our work suggests, however, that the irritability of crystalline deposits on natural surfaces should be ascertained whenever possible. DDT crystals from a wettable powder formulation produce very different results from the DDT in solution in Risella oil in the WHO papers and it is likely that similar differences will be observed with plaster, brick, stone and other building materials.

RÉSUMÉ

La méthode provisoire de détermination de l'irritabilité des moustiques adultes, telle qu'elle est recommandée par le Comité d'experts des Insecticides (1960), a fait l'objet d'une série d'épreuves en Ouganda. La technique originale, ou légèrement modifiée par les auteurs, ne s'est pas montrée entièrement satisfaisante dans les conditions offertes par le petit laboratoire de campagne du projet pilote d'éradication du paludisme en Ouganda. Il n'est possible, dans ce petit laboratoire, de régler ni la température, ni le degré d'humidité; la lumière ne peut être modifiée que par la position des stores; dans ces conditions, assez courantes sur le terrain, les résultats sont influencés par des facteurs tels que l'éclairement et le moment de la journée.

Parmi les moustiques utilisés dans cette expérience, *A. gambiae* est plus irrité par le DDT que *A. funestus*, *A. pharoensis* ou *Aëdes aegypti*. Cependant, toutes les souches examinées présentent d'importantes différences individuelles de sensibilité. Cette observation est particulièrement nette dans un essai portant sur trois générations successives d'une souche de laboratoire de *A. gambiae*. Les résultats obtenus au cours d'expériences utilisant des moustiques du même âge et nourris de la même manière indiquent une variance importante, qu'il s'agisse du moment du premier envol que du nombre de vols durant les 15 minutes d'observation.

On a pu constater qu'il y a moins de mouvements chez les moustiques témoins en utilisant du papier filtre noir au lieu du papier filtre blanc standard fourni par l'OMS; c'est là, certes, une amélioration de la méthode d'épreuve mais le remplacement du papier filtre standard de l'OMS serait certainement difficile. L'expérimentation a également porté sur l'emploi de blocs de boue; ceux-ci constituent un matériel bien supérieur à toute espèce de papier filtre, mais sa normalisation ne serait pas aisée. Cette expérience a montré que les cristaux de DDT sont moins irritants mais plus mortels que la solution en huile Risella dont est imprégné le papier filtre de l'OMS. Les résultats de déterminations de l'irritabilité ne doivent donc être appliqués que prudemment aux problèmes pratiques rencontrés sur le terrain.

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