Bull. Org. mond. Santé / *Bull. Wld Hlth Org.* 1958, **19**, 1053-1061

LABORATORY STUDIES ON THE BEHAVIOURISTIC RESISTANCE OF *ANOPHELES ALBIMANUS* IN PANAMA

A. W. A. BROWN, Ph. D.

Head of the Department of Zoology, University of Western Ontario, London, Ontario, Canada;
Formerly, Scientist (Biologist), World Health Organization

SYNOPSIS

The behaviouristic resistance of *A. albimanus* to DDT observed in populations on the Rio Chagres by Trapido in 1952 was re-investigated in 1958. Measurement of the excitatory effect of DDT in inducing flight showed that the mean excitation times for engorged adults from the Chagres populations of Gatuncillo and Santa Rosa were respectively significantly and almost significantly less than those for a population from an untreated area or for a laboratory population which had never encountered DDT. There was no difference in the susceptibility levels between the Gatuncillo, the untreated and the laboratory strains when they were confined to DDT deposits for one hour.

In 1952 it was observed that the numbers of adult *Anopheles albimanus* surviving residual house treatments of DDT in kerosene at the village of Gatuncillo on the Rio Chagres, Panama, were as much as seven times greater than the numbers which formerly survived the treatments in 1945 and 1946 (Trapido, 1952). The selective killing of engorged females brought about by a protracted resting-period on the DDT deposit evidently no longer occurred in 1952 as in 1946. Moreover, the adult mosquitos in the treated houses in 1952 were characterized by restlessness and a tendency to hover around lights. The same phenomenon was also observed at the village of Santa Rosa, some three miles down river from Gatuncillo, where spraying was commenced in 1947. When the susceptibility levels for DDT were subsequently assessed by the Busvine-Nash test (Trapido, 1954) adults from Gatuncillo and Santa Rosa were found to be no less susceptible than a standard laboratory colony of *A. albimanus*, collected in 1938 on the Atlantic coast of Panama and maintained thereafter at the Gorgas Memorial Laboratory, Panama, under insecticide-free conditions.

Trapido therefore concluded that the survival of *A. albimanus* in the treated houses " must be due to a behavious change, and not to any change in the intrinsic toxicity of DDT to this mosquito ", and suggested that the

change might be due to " the selection of a population hyperirritable to DDT ".

Since this instance appeared to be the most compelling example of behaviouristic resistance to insecticides by mosquitos, it was reinvestigated in 1958. The residual treatment had been continued twice a year since 1953, and the same lack of control in the houses had been noted. The study here reported was designed to compare the irritability to DDT of the Rio Chagres populations at Gatuncillo and Santa Rosa with that of an untreated field population from Rio Gatun, and with the Gorgas Laboratory colony. The tests were performed in April and May 1958 at the Juan Mina field laboratory on the Rio Chagres.

Methods

Engorged female *A. albimanus* were exposed singly to a deposit of DDT in oil solution on paper, and the time between their immediate settling and their subsequent taking flight as a result of the DDT was determined. A standard WHO pre-impregnated paper, measuring 12 cm by 15 cm was exposed in a vertical position. A hollow transparent plastic cone (WHO standard exposure chamber for wall bioassays), 8.5 cm in diameter and 5.2 cm deep, was placed against it and both were clamped to a white board with two large spring paper-clips. The assembly was illuminated by a tungsten-filament lamp at 40 foot-candles, and the observer sat behind the lamp. The mosquitos used had gorged on human blood two hours previously and their age was three days after emergence. They were introduced singly into the cone through an apical hole and their movements were noted until they took flight. All tests were performed between 7.30 and 10.30 p.m.

When introduced to control papers impregnated with oil alone, engorged female *A. albimanus* alight immediately and remain motionless for at least 60 minutes; four adults of the Gorgas Laboratory strain and three adults of the Gatuncillo strain did this, while one Gatuncillo adult took two minutes and five flights to settle down. In the settled position the mosquito rests principally on the metathoracic legs, which are spread wide, with the prothoracic claws holding the body from pivoting downwards into the paper. Unengorged mosquitos never rested for 60 minutes at a stretch. Accordingly in the experiments with treated papers, engorged adults were oberved for at least three minutes before a take-off was considered as an excitation time.

The mosquitos of the Gatuncillo, Santa Rosa and Rio Gatun strains were obtained from material collected in the field as larvae or pupae. Those of the Gorgas Laboratory strain were obtained from a subcolony at the Juan Mina field station. A total of 20 adults from each of the four populations was tested on each of three concentrations, namely, 1%, 2% and 4% DDT. The study therefore involved 240 adults, or a total uninterrupted observation time of 4618 minutes.

On a DDT-treated paper the first reaction of the mosquito is to rock the body on the legs, to move the mouth-parts, or to shift position slightly. Then the hind legs start waving and may be rubbed against each other, and one of the front legs may be lifted and rubbed against the proboscis. The mosquito may start to walk, for short or long periods, at speeds ranging from creeping to running. Occasionally, the wings are lifted and vibrated for a second or so. A normal excitation occurs when the metathoracic legs demand relief from the DDT, and the insect takes off. Occasional persistent individuals totter about until they lose their footing and fly.

Results

The times from contact with the paper to flight were recorded in minutes for each mosquito in the 12 experimental groups of 20, and the mean, median, standard deviation and standard error were computed (Table 1). One individual in the Gorgas, one in the Santa Rosa and two in the Rio Gatun

TABLE 1. FLIGHT EXCITATION TIMES IN MINUTES FOR 4 POPULATIONS OF *A. ALBIMANUS* IN PANAMA EXPOSED TO PAPER IMPREGNATED WITH 3 CONCENTRATIONS OF DDT IN OIL

Concentration of DDT	Mean	Standard deviation	Coefficient of variation	Standard error	Median	Longest Time
Gorgas Laboratory						
1 %	41.1	22.5	55 %	5.2	40.2	90.0
2 %	22.7	9.1	40 %	2.1	19.1	47.0
4 %	17.2	12.3	72 %	2.8	15.1	50.7
Gatuncillo						
1 %	25.2	15.9	65 %	3.6	24.0	53.1
2 %	10.7	6.5	61 %	1.5	12.6	23.8
4 %	7.8	7.8	99 %	1.8	5.8	28.6
Santa Rosa						
1 %	26.9	26.9	100 %	6.2	14.7	90.0
2 %	11.4	8.2	72 %	1.9	11.8	31.5
4 %	10.8	9.9	92 %	2.3	8.0	31.2
Rio Gatun						
1 %	27.7	23.8	86 %	5.5	21.9	90.0
2 %	16.6	17.1	103 %	3.9	10.2	75.7
4 %	12.8	8.6	67 %	2.0	11.0	31.0

strains remained resting on the paper impregnated with 1% DDT until observation was terminated after 90 minutes. It will be seen that there is a high variance between the individual flight excitation times, the coefficients of variation ranging from 40% to 103%. However, this variance was less than that observed by Hadaway & Barlow (1953) with *Anopheles stephensi* on deposits of DDT crystals at 150 mg per square foot (or about 1.5 per m^2), where three lots of 20 mosquitos showed an average excitation time of 3.2 minutes and an average coefficient of variation of 139%. It was natural to consider that the amount of engorgement, the exact time after feeding and the general vigour of the mosquito might be factors in this variance, but general experience throughout the study did not give any definite indication in any direction.

The figures in Table 1 show that the mean excitation times for all four strains decrease from 1% through 2% to 4% DDT papers, and the median values show the same relationship. The excitation times for the Santa Rosa and Gatuncillo populations are lower than the corresponding values for the Rio Gatun population, and all three are considerably lower than those

TABLE 2. FREQUENCY DISTRIBUTION OF FLIGHT EXCITATION TIMES AND EXCITATION TIMES FOR MOTION OR CLEANING OF THE FORE OR HIND LEGS

Concentration of DDT	Number in each time class				Leg movements	
	< 5	5-12	12-20	> 20	average time (minutes)	number showing movement
Gorgas Laboratory						
1%	0	1	3	16	27	11
2%	0	1	10	9	12	15
4%	1	6	9	4	9	16
Gatuncillo						
1%	3	2	3	12	20	5
2%	6	3	10	1	9	14
4%	9	9	0	2	5	8
Santa Rosa						
1%	4	5	3	8	24	10
2%	7	3	7	3	9	7
4%	4	10	4	2	8	12
Rio Gatun						
1%	0	5	4	11	15	11
2%	2	10	4	4	11	9
4%	2	11	4	3	5	17

for the Gorgas Laboratory strain. The times to the first onset of leg movements follow the same general relationship with strain and concentration (Table 2). The frequency distribution of individuals into excitation time classes also changes, so that as the concentration increases the numbers of long-resters decrease and the numbers of short-resters increase, and the same change occurs as one passes from the Gorgas through the Rio Gatun to the Gatuncillo and Santa Rosa populations.

When each mosquito had been excited to fly, it was removed, placed in a waxed cardboard cup, and kept for 24 hours with water available on a cotton wool pad. The mortalities shown after this period (Table 3), were surprisingly low, and they tended to be higher at the higher concentrations; moreover, the Rio Gatun population showed the greatest death-rate.

TABLE 3. MORTALITIES OF ADULTS OF *A. ALBIMANUS* IN PANAMA AFTER EXPOSURE TO DDT EITHER UNCONFINED OR CONFINED FOR 1 HOUR, AND MEDIAN LETHAL CONCENTRATIONS FOR THE LARVAE

Concentration of DDT	Number of adults dead after 24 hours' observation		Larval LC_{50} (parts per 1000 million)
	exposed and excited to fly	confined for 1 hour	
Gorgas Laboratory			
1%	2	7	
2%	5	16	
4%	5	20	2.2
Gatuncillo			
1%	4	—	
2%	3	—	
4%	6	—	—
Santa Rosa			
1%	4	10	
2%	2	17	
4%	6	20	1.3
Rio Gatun			
1%	5	9	
2%	6	—	
4%	11	—	0.8

Although there is no doubt that much of this mortality was due to causes not connected with the DDT, it was three times higher in the long-resters than in the short-resters for the 2% and 4% DDT groups.

Groups of 20 mosquitos were confined in lots of six or seven to the papers impregnated with 1%, 2% or 4% DDT in 3-inch by 1-inch (7.5×2.5 cm) glass vials for one hour, and were observed for mortality 24 hours later, following the method of Busvine & Nash (1953). The results (Table 3) show no significant differences between the Santa Rosa, the Rio Gatun, or the Gorgas Laboratory strains. Trapido (1954) had shown that by this test there was no difference between the Gatuncillo, Santa Rosa and Gorgas Laboratory populations in 1953.

Larval tests for susceptibility, following the standard WHO method, were performed on 116 larvae of the Rio Gatun, 326 of the Santa Rosa, and 934 of the Gorgas Laboratory strains, all of which were in the late IIIrd stage. Since *A. albimanus* larvae are characteristically very susceptible to DDT, it became necessary to dilute the lowest test concentration supplied, that at 0.004 parts per million (p.p.m.), to successive concentrations of 0.002, 0.001 and 0.0005 p.p.m. The minimal LC_{100} for the Gorgas Laboratory and Santa Rosa strains was 0.02 p.p.m., whereas that for the Rio Gatun population was 0.002 p.p.m. But there was little difference between the LC_{50} levels of the three strrains, which were respectively 2.2, 1.3 and 0.8 parts per 1000 million (Table 3).

The statistical significance of the differences between the mean values for adult excitation times, between DDT concentrations within a given strain, and between pairs of strains for a given concentration, was estimated by calculating the " t " values (Table 4). The differences between the mean values for 2% as against 1% DDT are significant (at the 5% probability level) for three of the four strains. Those for 2% as against 4% DDT are not significant for individual strains, although it is reasonable to assume that the use of greater numbers would have indicated the statistical significance of a difference that is logically to be expected. Only the Gorgas Laboratory strain showed significant differences from the other populations, which occurred in four out of the nine group comparisons involved with this strain.

The " t " values evaluating inter-strain differences are less in the 1% than in the 2% and 4% DDT concentration classes, and are partially attributable to the occasional mosquito resting on 1% DDT for at least 90 minutes and thus increasing the variance tremendously. If the values of " t " for the 2% and 4% DDT classes of comparison are considered, it is found that between the Gatuncillo and Rio Gatun strains they are 1.4 and 1.9 respectively. Although neither of these figures indicates a significant difference at the 5% level (for which a " t " value of 2.1 is required), they do indicate that if the 40 mosquitos involved had been tested at a single concentration instead of the two, significance would have been attained.

When an analysis of variance was performed to compare the two strains from Gatuncillo and Rio Gatun at the two concentrations of 2% and 4% DDT together, it was found that the value of F between strains was 5.16

TABLE 4. VALUES OF "t" FOR COMPARISONS BETWEEN MEAN VALUES OF THE 4 POPULATIONS AT THE 3 CONCENTRATIONS

Between concentrations	1% *versus* 2%	2% *versus* 4%
Gorgas Laboratory	3.9	1.6
Gatuncillo	3.8	1.3
Santa Rosa	2.9	0.6
Rio Gatun	1.7	0.9

Between concentrations	*Gatuncillo*	*Santa Rosa*	*Rio Gatun*
1% Gorgas Laboratory	2.6	1.8	1.8
Gatuncillo			0.1
Santa Rosa		0.1	0.4
2% Gorgas Laboratory	4.8	4.1	1.4
Gatuncillo			1.2
Santa Rosa		0.3	1.4
4% Gorgas Laboratory	2.9	1.8	1.3
Gatuncillo			0.7
Santa Rosa		1.1	1.9

(for 1 and 77 degrees of freedom), which indicates that the activation time of the Gatuncillo population was significantly lower than that of the Rio Gatun population. A second analysis of variance was performed using the logarithms of the activation times, a plot of their cumulative frequency distribution on probability paper having shown that it is more linear if the time is on a logarithmic rather than an arithmetic scale. This analysis included the Santa Rosa as well as the Gatuncillo and Rio Gatun strains, at the two concentrations of 2% and 4% DDT. It showed that the variation between strains was significant, almost at the 1% probability level, the F value being 4.39 (for 1 and 116 degrees of freedom).

The means of the logarithms of the activation times at 2% and 4% DDT, taken together, were calculated for each strain, and the differences of the Gatuncillo and Santa Rosa means from the Rio Gatun mean were derived by subtraction. When compared with the amount of difference necessary for significance, as calculated by either Scheffé's or Tukey's method (Scheffé, 1953), the difference between the Gatuncillo and Rio Gatun strains was significant at the 5% level and almost at the 1% level. The difference between the Santa Rosa and Rio Gatun strains was slightly short of significance at the 5% level.

Discussion

The most striking feature of the results is the lack of DDT-excitability of the Gorgas Laboratory strain, which showed activation times approximately twice as long as the field strains. How much of this difference was due to the difference in larval nutrition is unknown; the Gorgas larvae bred on yeast and vegetable meal were white, whereas the field larvae feeding on unicellular plants were olive-green and white-banded. But it is probable that the laboratory rearing of this (Gorgas Laboratory) colony for 20 years effected a change towards inexcitability, thus indicating that DDT-excitability is a character susceptible to selection. Another feature is the relative inexcitability to DDT of *A. albimanus* as a species; the mean excitation times found were as a whole greater than the means of 8 and 10 minutes shown respectively by *A. gambiae* and *A. funestus* on DDT deposits in the field (Davidson, 1953) and very much greater than the mean of 3.2 minutes found for a laboratory colony of *A. stephensi* (Hadaway & Barlow, 1953).

The results also indicate that the populations on the Rio Chagres are on the average more DDT-excitable than an untouched population at Rio Gatun, the difference being significant for Gatuncillo and almost significant for Santa Rosa. However, since the differences are small, and the frequency curves of the different populations overlap to a very great degree, it is difficult to consider that they account for the behaviouristic resistance in its entirety; nonetheless it is probably true that the small laboratory-observed differences in time to first flight will be compounded in the many successive landings and take-offs in a sprayed house in the field.

It is noteworthy that whereas the Rio Gatun mosquitos were no more susceptible than the Rio Chagres populations when confined to DDT surfaces in glass vials, they did show a higher mortality rate when the confinement was eliminated by taking them away as soon as they had been activated by the DDT to fly. It is just possible that tests of the Busvine-Nash type do not tell the whole story, and that differences in real susceptibility are sometimes obscured. Such tests depend for their results not only on the real susceptibility of the mosquito, but also on its degree of activity caused by mutual activation when confined with others in the tube; when only a single mosquito is tested, the mortality is low, presumably because it remained at rest when exposed to the deposit (Raffaele, Coluzzi & Zulueta, 1958). Studies performed in the spacious environment of experimental huts should show these differences between the populations in terms of mortality after exposure to DDT-treated surfaces.

ACKNOWLEDGEMENTS

Thanks are due to Dr J. P. Duret, Pan American Sanitary Bureau, for his help and advice during this investigation, to Dr C. M. Johnson, Director of the Gorgas Memorial Laboratory, for his kindness in allotting the Juan Mina Laboratory for this study, and to Mr. Henry van Horn, for his invaluable services in the field.

RÉSUMÉ

En 1952, on avait signalé un changement de comportement d'*Anopheles albimanus* à Panama, à la suite du traitement par le DDT attribué par l'auteur qui le décrivait à la sélection d'une population hyperirritable, sous l'effet du DDT. Le fait était si important qu'il demandait confirmation. Des essais de laboratoire ont été entrepris en 1958, afin de comparer l'irritabilité au DDT de populations anophéliennes des zones traitées, de celles de zones non traitées, et de souches de laboratoire, en déterminant le temps d'excitation, soit l'intervalle séparant le contact avec l'insecticide de l'envol.

La souche de laboratoire, entretenue depuis 20 ans, est extrêmement peu irritable, deux fois moins que les souches sauvages. *A. albimanus* est une espèce relativement peu excitable. Le temps d'excitation dépassait de beaucoup les 8 et 10 minutes trouvées pour *A. gambiae* et *A. funestus* respectivement, et les 3,2 minutes pour *A. stephensi*. La population anophélienne d'une zone traitée (Rio Chagres) était dans l'ensemble plus excitable qu'une population de zone non traitée (Rio Gatun), de façon significative ou presque significative. Il ne semble toutefois pas que ces différences puissent se traduire par un changement de comportement.

La mortalité des populations non préalablement exposées au DDT et celle des populations des zones traitées était la même lorsqu'on laissait les moustiques au contact des surfaces traitées dans des flacons de verre. Elle était plus élevée dans le premier groupe, lorsqu'on éloignait les moustiques, sitôt après qu'ils aient quitté la zone traitée. D'autres éléments de sensibilité interviennent peut-être, que la méthode Busvine & Nash ne permet pas de déceler. Un autre facteur à prendre en considération est l'activation collective, qui se produit lorsque plusieurs anophèles sont réunis dans le même tube. La mortalité, déterminée sur des individus isolés, se reposant après exposition à l'insecticide n'est pas la même que dans un groupe de moustiques, soumis à une sorte d'activation mutuelle. Il y aurait lieu de poursuivre ces études sur des populations de moustiques, dans des huttes expérimentales, afin de mettre en évidence ces différences dans le taux de mortalité après exposition au DDT.

REFERENCES

Busvine, J. R. & Nash, R. (1953) *Bull. ent. Res.*, **44**, 371
Davidson, G. (1953) *Bull. ent. Res.*, **44**, 231
Hadaway, A. B. & Barlow, F. (1953) *Bull. ent. Res.*, **44**, 255
Raffaele, G., Coluzzi, M. & Zulueta, J. de (1958) *Bull. Wld Hlth Org.*, **18**, 464
Scheffé, H. (1953) *Biometrika*, **40**, 87
Trapido, H. (1952) *Amer. J. trop. Med. Hyg.*, **1**, 853
Trapido, H. (1954) *Bull. Wld Hlth Org.*, **11**, 885