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A METHOD FOR FIELD DETECTION OF ADULT-MOSQUITO RESISTANCE TO DDT RESIDUES*

R. W. FAY, Ph.D.
Chief, Insecticide Section, Technical Development Laboratories
J. W. KILPATRIK, M.S.
Control Methods and Evaluation Section, Technical Development Laboratories
R. L. CROWELL, M.S.
Insecticide Section, Technical Development Laboratories
K. D. QUARTERMAN, M.P.H.
Chief, Technical Development Laboratories

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SYNOPSIS

A test procedure involving simply-constructed, portable test-equipment has been developed for detecting resistance to DDT residual applications by adult mosquitos. The procedure involves timed exposures of adult female mosquitos to DDT residues in chambers made of paper and screen wire, and the determination of the subsequent 24-hour mortalities. Data on mortality figures for *Anopheles quadrimaculatus* Say as influenced by test-chamber preparation, exposure period, insect age, and testing and holding temperature and relative-humidity combinations are given. Statistical procedures used in establishing a base line and in determining the sensitivity of the method for detecting resistance are outlined.

Residual application of DDT is one of the methods of attack against mosquitos transmitting malaria, yellow fever, filariasis, dengue, and other mosquito-borne diseases. Since such control operations have been world-wide, the areas under treatment are often quite distant from sources of insecticides, and large quantities of insecticide must be ordered well in advance of the proposed usage dates. In view of this problem, early detection of DDT resistance in mosquitos is desirable to allow alteration of operational plans. Therefore, a test procedure for detecting DDT resistance in adult mosquitos has been developed with consideration of (1) the availability and cost of test materials, (2) the ease of equipment construction by field personnel, (3) the weight and bulk of apparatus, and (4) the simplicity of the testing procedure.

* From the Technical Development Laboratories, Communicable Disease Center, Public Health Service, US Department of Health, Education, and Welfare, Savannah, Ga., USA

Equipment and Procedure

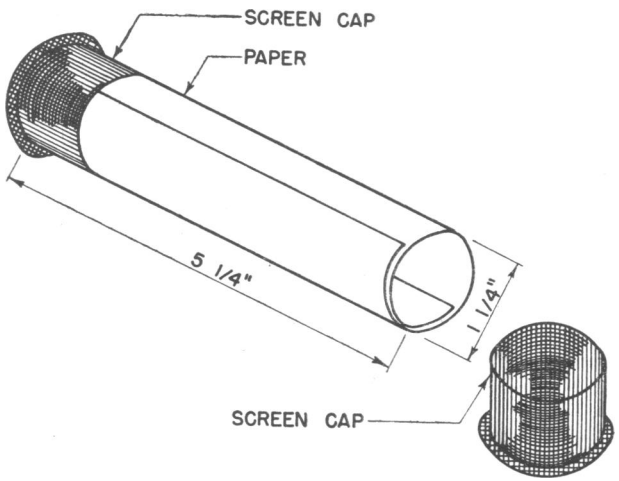
The equipment required for testing is as follows : (1) DDT, either technical grade or water-wettable, in sufficient quantity for initial, base-line, and resistance evaluations; (2) xylene, as a preferred DDT solvent, although other solvents may be used; (3) screen wire or other suitable materials for making end caps for exposure- and holding-cages; (4) a stock of standard absorbent paper which may be cut into test-sheets approximately 5 inches × 8 inches (13 cm × 20 cm) for making the walls of the exposure chambers; (5) a shallow container slightly larger in dimensions than the sheets of test-paper; (6) a pair of forceps for handling the test-papers; (7) a board with small nails at about 2-inch (5-cm) intervals to support test-papers in a horizontal position while drying; (8) an aspirator tube for handling test-insects; and (9) suitable food materials for the test-insects.

Letter-size mimeograph paper, 8 inches × 10.5 inches (20 cm × 27 cm), was used in all described tests. Each sheet was cut in half to form test-papers 5.25 inches × 8 inches (13 cm × 20 cm). Groups of 30 papers were numbered consecutively on one margin. Using technical-grade DDT, 1% w/v solutions in xylene were prepared. With water-wettable DDT, the required amount based on the DDT content of the powder was weighed in a container. Xylene was then added so that, with the specific volume of DDT taken as 0.655, a 1% w/v solution would be obtained, and the xylene and powder were shaken for 1 minute. After an interval of not less than 5 minutes, the clear xylene was decanted off. Essentially complete extraction of the DDT was effected.

A shallow enamelware tray, 8.5 inches × 6 inches × 1.5 inches (22 cm × 15 cm × 3.8 cm), was partially filled with 150 ml of the DDT-xylene solution. Each test-paper, held at the numbered margin, was submerged and pulled through the solution. The sheet was allowed to drain vertically for 10 seconds and then laid horizontally on the nail points for 24-72 hours at room conditions before use. Test-chambers were formed by rolling each treated paper with the numbered end innermost into a cylinder 1.25 inches (3 cm) in diameter and 5.25 inches (13 cm) in length. The ends of the cylinder were covered with screen-wire caps. Holding-chambers were fashioned in exactly the same manner except that untreated papers were used. The chamber and screen-wire end caps are shown in fig. 1.

Twenty adult female mosquitos were used in each replicate. They were collected in a glass-tube aspirator, 18 inches (46 cm) long and half an inch (13 mm) in diameter, and blown gently into a test-chamber. At the end of the exposure period, one cap was removed from the test-chamber and one cap from the holding-chamber. The open ends of the cylinders were immediately placed together, the mosquitos were gently blown into the holding-chamber, and the caps were replaced. The testing-chamber was then discarded. During the holding period, the mosquitos were

FIG. I. CHAMBER AND SCREEN-WIRE CAPS USED IN DDT-RESISTANCE DETERMINATIONS



furnished with a honey-water solution on cotton. Mortality determinations were made 24 hours after the exposure period.

Results

Basic data on the sensitivity of the test procedure were obtained using normal insectary-reared female *Anopheles quadrimaculatus* Say adults. With this species, test-papers treated with a 1% DDT-xylene solution were found satisfactory. No marked differences were found in biological determinations using, (1) treated papers that dried 1-7 days before testing, (2) the 1st, 15th, or 30th papers from a series dipped in the same 150 ml of DDT-xylene solution, or (3) test-cylinders made by rolling the papers in different ways.

Exposure conditions necessary to produce from 70% to 90% mortality of normal adult female mosquitos were desired so that measurement of any incipient resistance would be possible in the more sensitive range of the test procedure. Preliminary evaluations with both sexes using exposure periods of 1, 5, 15, and 30 minutes gave high 24-hour mortalities of the adult males, but only reached 50% mortality of the females with the 30-minute exposures. The final exposure period adopted, 45 minutes, produced an average of 70% mortality of the adult females at 24 hours.

Using exposure periods of 45 minutes, the effects of the testing and holding temperature and humidity conditions on mortality were evaluated in three categories : (1) 75°F (23.9°C) with 70% relative humidity;

TABLE I. MORTALITY OF ADULT FEMALE NON-RESISTANT ANOPHELES QUADRIMACULATUS *

Type of data	Number of replicates	Mean mortality	Mean values below these necessary for significance based on					
			50 replicates		20 replicates		10 replicates	
			0.05	0.01	0.05	0.01	0.05	0.01
Three-day-old								
75°F (23.9°C) at 70% RH [a]	50	68.2	64.1	62.6	61.4	58.8	57.6	52.8
80°F (26.7°C) at 70% RH	50	78.1	74.6	73.3	72.4	69.8	68.8	64.4
88°F (31.1°C) [b] at 20%-50% RH	50	94.2	90.7	89.3	88.0	85.3	84.1	78.5
72°-95°F (22.2°-35°C) [c] at 20%-70% RH	150	81.6	76.2	74.3	72.4	68.8	67.1	60.1
Variable age								
75°F (23.9°C) at 70% RH	50	73.0	70.3	69.5	68.6	67.0	66.3	61.5
80°F (26.7°C) at 70% RH	50	81.2	76.4	74.7	73.0	69.9	68.4	62.3
88°F (31.1°C) [b] at 20%-50% RH	50	97.8	96.0	95.4	94.3	92.7	92.6	89.5
72°-95°F (22.2°-35°C) [c] at 20%-70% RH	150	86.1	81.1	79.4	77.6	74.2	72.5	65.7
All ages								
72°-95°F (22°-35°C) [c] at 20%-70% RH	300	84.0	78.7	76.8	75.0	71.4	69.8	62.8

* Mean 24-hour mortality after 45-minute exposures to DDT residual deposits from a 1% DDT-xylene solution on paper, and calculated mean values necessary in a second series of tests to indicate possible resistance to DDT in *A. quadrimaculatus* (20 per replicate) are shown.

[a] RH = relative humidity

[b] Temperatures ranged from 72°F to 95°F (23.9°C to 35°C)

[c] Combined data for age-group designated

(2) 80°F (26.7°C) with 70% relative humidity; and (3) variable temperature ranging from 72°F (22.2°C) to 95°F (35°C) (mean 88°F (31.3°C)) with relative humidities of 20%-50%. In each category two age-groups of adult female mosquitos were tested—namely, 3-day-old adults, and adults ranging from 1 to 18 days old. Fifty replicates with 20 adult female mosquitos were run for each of the six combinations of temperature and humidity and age-group to determine biological variations under different degrees of controlled factors.

In the analysis of the results obtained the following steps were taken :

(1) since the data from each replicate was expressed as percentage mortality, the percentage figures were subjected to sine transformations before further calculations were made;

(2) the standard deviation and the mean error were calculated for each series of replicates;

(3) based on the deviations shown by the initial 50 base-line replicates, the mean values which would be necessary for a significant difference indicating incipient resistance in a second series of 50, 20, or 10 replicates were computed (table I).

As can be seen in the table, higher testing and holding temperatures produced higher mortalities and the variable-age mosquitos were somewhat more susceptible than were the 3-day-old adults. These evaluations were made to demonstrate the effect of insect age and of temperature in view of the fact that in practical field use of the method it will probably be impossible to control these factors.

Discussion

In view of the effects of temperature upon mortality in these tests, it appears desirable that, wherever possible, the testing-sites for field evaluations should be located so as to offer the least range of variation in daily and seasonal temperature. Humidity changes should be as low as possible. It would also be desirable to collect larvae or pupae in the field and rear them to adults, so that the age of the adults would be controlled in the tests. Lacking these advantages, the establishment of base-line susceptibility of the local mosquitos should be formulated by a series of replicates on successive days to randomize somewhat any daily variations in temperature, humidity, or insect age.

The procedure described is based upon tests with *A. quadrimaculatus* adults at Savannah, Ga., USA, and it is recognized that modifications in evaluation conditions must be made for use of the method against other species and in other geographical areas. The equipment described, such as the type of DDT, the DDT-solvent, the paper for the chamber walls, the type of cover caps, and the cage size, may be varied to suit local conditions as long as the same conditions are utilized throughout the evaluations for any given species in any given locality.

In general, the procedure may be divided into three phases :

(1) the initial tests to determine exposure conditions which will produce between 70% and 90% mortality of the local adult females of each mosquito species in each locality for which resistance data are desired;

(2) the establishment of a base-line series for each species in each locality under these exposure conditions to determine the biological variation of the method and to allow prediction of significant differences indicating possible resistance; and

(3) the performance of test-replicates at subsequent time intervals for comparison with the original base-line in evaluating resistance.

Twenty adult females were used in each reported replicate since each death gave an increment of 5% mortality. With 25 or 33 adult females, increments of 4% or 3%, respectively, might be obtained. These numbers

of mosquitos could have been tested adequately in the standard chambers described. It is felt, however, that, with as many as 50 adult mosquitos per replicate necessary for increments of 2%, crowding during the exposure and the holding periods would influence results. In laboratory tests, with adults aspirated from a cage of mixed sexes, 3 or 4 adult males were often included inadvertently with the 20 test-females. These numbers of additional mosquitos did not materially influence the mortality figures. In field collections, the occurrence of adult males with the females is relatively minor, but the inclusion of adult females of other species might represent a similar problem.

Since the replicate variation of the base-line series furnishes the basis for estimating significant differences for subsequent series evaluations, it is recommended that at least 50 replications be used in determining the base line. With the use of fewer replications in the base-line series, greater differences in subsequent series would be necessary for significance.

Since DDT has been in use for several years in many areas and the possibility exists that resistance to DDT may already be developing in some mosquito species, it would be desirable to obtain, wherever possible, test-mosquitos which have never been exposed to DDT, or have received a minimum of exposure, for use in the initial and base-line tests discussed earlier. This would permit the detection of any DDT-resistance which may be currently developing in any given treated area.

It should be recognized that the present evaluation method is valid only in detecting increased ability of the insects to withstand contact with DDT residual deposits. Resistance which might arise from avoidance of DDT residual applications through changes in behaviour patterns, or resistance which might arise from increased sensitivity to the excitant action of DDT leading to reduction in contact periods, would not be reflected in the proposed procedure.

The method described for the determination of DDT-resistance might be applied to other types of residual insecticides, provided these chemicals do not have a marked fumigant effect. In the case of the more volatile insecticides, it is felt that the fumigant action would be over-emphasized in the procedure described here.

RÉSUMÉ

Les auteurs ont mis au point une méthode permettant de déceler la résistance de moustiques adultes au DDT à action rémanente. Un appareil simple, aisément transportable, a été construit à cet effet. Il est composé d'un cylindre de feuilles de papier absorbant, imprégné de DDT, fermé par deux capuchons de treillis métallique. Les femelles adultes séjournent dans ces cages durant un laps de temps donné; puis on détermine le pourcentage de mortalité au cours des 24 heures suivantes.

Les expériences faites par les auteurs avec des exemplaires d'*Anopheles quadrimaculatus* âgés de 3 jours ont montré qu'un séjour de 45 minutes, provoquant une mortalité de 70% environ, assure les meilleurs résultats.

La mortalité est influencée par la température et l'humidité relative ainsi que par l'âge des moustiques. Il est préférable d'utiliser des moustiques d'élevage, dont l'âge est exactement connu.

Les auteurs exposent les méthodes statistiques appliquées pour fixer une base à partir de laquelle on peut, pour chaque espèce de moustique et chaque localité, établir la variation biologique de la méthode et déceler les différences significatives pouvant traduire une résistance des moustiques au DDT.