WORLD HEALTH ORGANIZATION
ORGANISATION MONDIALE DE LA SANTÉ

VBC/EC/80.1
ENGLISH ONLY

EXPERT COMMITTEE ON
RESISTANCE OF VECTORS
OF DISEASE TO PESTICIDES

Geneva, 3-9 June 1980

INSECTICIDE RESISTANCE IN BLACKFLIES

by

J. Mouchet[1]

1. INTRODUCTION

Apart from a document presented by Guillet et al. in 1977, all the information on insecticide resistance in blackflies dates from before 1970 and has already been reported by Brown & Pal (1973). Most of the authors alerted by anti-blackfly treatment failures have compared the larval populations of the treated rivers with those of untreated rivers. From an observed reduction in susceptibility they have deduced the presence of resistance. But since each experimenter has used a different method it is impossible to check their findings against international standards.

Accordingly, before comparing the data obtained we will review the different techniques utilized.

2. METHODOLOGY FOR EVALUATION OF THE SUSCEPTIBILITY OF BLACKFLY TO INSECTICIDES

2.1 Larvae

2.1.1 Description of techniques utilized

Thompson (1975) made a critical review of the various methods proposed by Lea & Dalmat (1954), Muirhead-Thompson (1957), Jamnback (1962), Jamnback & West (1970) and Ovazza & Valade (1963) (Table 1). He himself proposes a laboratory technique adaptable to field use based on exposure for 30 minutes to different concentrations of insecticides, the medium being aerated with an aquarium pump. The larvae are placed in bags made of netting and then kept under observation for 20 hours in "aerated" water in the case of a laboratory test and in the current of the river in the case of a field experiment. This method differs little from the one proposed by WHO (Anon, 1975b) which involves an exposure time of 30 minutes in pre-oxygenated but not aerated water followed by 24 hours' observation in aerated water. It is specified that the method is not suitable for use with Simulium damnosum.

All the above-cited authors recommended an observation period in aerated water or running water of 20 to 24 hours. But Ovazza & Valade (1963) noted that mortality among larvae kept under observation in stagnant water differed little from that among larvae placed in aerated water. In the light of that observation Quélennec & Vervent (1970) adapted to blackfly larvae the method utilized for evaluating the susceptibility of mosquito larvae: 24 hours' exposure in pre-oxygenated water and immediate reading. The results were excellent when the tests were performed in the laboratory: mortality among the controls was below 2%

[1] Overseas Scientific and Technical Research Office, Central Scientific Services, 70 route d'Aulnay, 93140 Bondy, France.

and the regression curves were statistically satisfactory. However, under field conditions in Africa the application of this technique posed some problems. Without air-conditioned premises the high midday temperatures - over 25°C - caused high mortality among the controls. Mouchet et al. (1977) applied the same principles but reduced the exposure time to three hours, in pre-oxygenated water, after determining that the product obtained by multiplying contact time by concentration was constant as in tests performed on mosquito larvae. The reading and mortality count were done as soon as the exposure time was up, without any observation period. The results obtained were statistically satisfactory and mortality among the controls was below 2%. The tests could be performed right by the breeding site, so that no transport was needed, and in the morning or the evening when outside temperatures allowed a good rate of survival of the larvae for three or four hours.

The shorter the exposure time, the higher the concentration needed to obtain 100% mortality. Blackfly larvae having relatively low susceptibility to contact insecticides, it soon becomes necessary to use concentrations higher than the solubility of the insecticide. The dosage/mortality relationship then no longer necessarily conforms to the laws constituting the basis of the entire methodology in use. Suzuki et al. (1963), who had reduced the exposure time to 10 minutes, had to use concentrations of 5000 ppm of DDT, which are in fact suspensions.

By lengthening the contact time, Quélennec & Vervent (1970) and later Mouchet et al. (1977) were able to use much lower concentrations, which make results reproducible.

Note that Raybould in 1966 proposed a specific test for blackfly larvae of the Simulium neavei group that attach themselves to crabs.

2.1.2 Problems in applying the tests

2.1.2.1 Utilization of aerated or stagnant water

In order to maintain rheophilic blackfly larvae in the best possible conditions for survival, most authors have proposed that they be placed in aerated or running water at least during the observation period. This creates constraints for experimenters in the field. Moreover, immersion of larvae in river water can entail risks of contamination when the entire hydrographic system is under insecticide treatment. This is so, for example, in the zone covered by the Onchocerciasis Control Programme in the Volta River Basin (OCP).

However, Simulium larvae survive well in stagnant water, pre-oxygenated for 6 to 24 hours according to the climatic conditions. This length of time is sufficient for performing the considerably simplified field test proposed by Mouchet et al. (1977).

This method, applied by Guillet et al. (1977) in the whole of the OCP zone, has given reproducible results, even under very difficult field conditions. It has enabled the susceptibility both to DDT and to temephos of the various species of the S. damnosum complex to be evaluated.

Performing the work near the breeding sites eliminates the problems of transporting the larvae to the laboratory, which may be several hours' drive away. It is desirable that the method recommended by WHO be applicable in difficult field conditions and as far as possible free the investigator from the constraints of the laboratory.

2.1.2.2 Criteria of mortality

The criterion of larval mortality adopted by WHO is absence of reaction to a pinprick. But alongside properly dead larvae there are in all tests some moribund larvae which have lost all spontaneous movement but react to the prick. Mouchet et al. (1977) considered that the mortality figure should include dead and moribund larvae. The ratio $\frac{LC_{95}}{LC_{50}}$ is then

smaller. Hence the slope of the regression lines is steeper and the development of resistance is more easily detected at the outset.

2.1.2.3 Choice of larval stages

In the case of Simulium damnosum, larvae of instars 6 and 7 are 5 to 10 times less susceptible to temephos than larvae of instars 4 and 5 (Mouchet et al., 1967). It is therefore recommended that the latter be used.

2.1.2.4 Species identification

It is of course essential to work with batches containing only one species. Identification of specimens is, however, often difficult when collecting the material and preparing for the test. Their identity must therefore be checked after the test is completed. Even so, it is sometimes very difficult during surveys to distinguish species belonging to a single complex, e.g. Simulium damnosum s. st. and S. sirbanum. Hitherto differences in susceptibility between species of the complex S. damnosum s.l., though constant, have been slight. The LC_{95} values for DDT of S. soubrense and S. sanctipauli are at most twice as high as those of S. damnosum s. st. and S. sirbanum (Guillet et al., 1977). These differences cannot produce any fundamental error of interpretation but it is nevertheless well to take them into account as far as possible.

2.1.2.5 Other technical problems

The optimum test temperature of course varies according to the species and especially the ecological conditions. In stagnant water at above 25°C there is high mortality among S. damnosum. Moreover, the activity of some insecticides varies with the temperature. It is therefore necessary to determine in each region the standard conditions for performing the tests.

Finally, the larvae being fragile, their manipulation with tweezers must be done with care so as to obviate mortality among the controls, which should be below 10%.

2.1.3 Recommendations

There is an urgent need for WHO to undertake an evaluation of the various methods and propose the adoption of one or more protocols. A technique suited to S. damnosum in Africa is not necessarily valid for holarctic blackfly. Without the same kind of standardization adopted for mosquitos it will not be possible to compare the findings of different experimenters.

Then, when a methodology has been adopted, it would be desirable to establish diagnostic dosages for the different insecticides so that survey procedures can be simplified. Mouchet et al. (1977) proposed 0.25 ppm as diagnostic dosage for temephos in S. damnosum s.l., at a temperature of 20-25°C and with an exposure time of three hours.

2.2 Adults

WHO (Anon, 1975a) has proposed a method very close to the one used for testing adult mosquitos. The insects are exposed for one hour on slips of paper impregnated with insecticide in plastic tubes. Then they are placed under observation for 24 hours, at the end of which the mortality is noted.

To judge by the absence of results in the literature, very little recourse has been had to this method.

3. RESISTANCE IN BLACKFLIES

It was in Japan in 1963 that Suzuki et al. reported for the first time a 10-fold resistance to DDT and a 2.6-fold resistance to lindane among the larvae of Simulium aokii in the Jurume river. They had been alerted by the failure of anti-blackfly treatments with these insecticides after a decade of campaigns. They based their conclusions on a comparison between specimens obtained from the river Jurume and from the river Hanno, which had never been treated. However, the technique that was used by those authors (see 2.1.1 above) is not very satisfactory.

By the same comparative method Asahina et al., in 1966, demonstrated in S. ornatum levels of resistance in the region of 10-fold to DDT, diazinon and fenthion (Nagano, Prefecture). These are the only cases of resistance to organophosphorus insecticides reported up to now.

In Canada, West (1967) noted a reduction in susceptibility to DDT by a factor of 10 in S. venustum at Petit Bras, Quebec.

In the United States of America, in the north of New York State, Jamnback & West (1970) reported a reduction in susceptibility to DDT by a factor of 5 to 10 in S. venustum and S. fuscum after 20 years of treatments. Those authors very rightly stressed the slowness with which resistance develops in cold climate species, which have few generations per year (often between 1 and 3).

In Ghana, during the building of the Akosombo Dam, the Volta River was treated with DDT at a dosage of 0.5 ppm/30 minutes to protect the workers from blackfly bites. In 1968, Kuzoe & Noamesi (in Brown & Pal, 1973) recorded treatment failures. The tests performed by the WHO method at that time gave a LC_{50} of 0.04 ppm for S. damnosum after 30 minutes' exposure compared to 0.005 ppm for a susceptible strain. However, the species remained susceptible to dieldrin, which was then employed.

At Kainji, in Nigeria, Walsh (1970) reported that between 1961 and 1968 the doses of DDT had had to be doubled to achieve good control of S. damnosum.

In Upper Volta and the Ivory Coast, after some years' control of S. damnosum with DDT, the staff in charge of the treatments reported in 1970 that the effectiveness of that insecticide was diminishing. In 1975, the zone being treated with temephos, the problem was eliminated before any evaluation of a putative resistance had been made.

However, in 1976, when monitoring the susceptibility of S. damnosum in the zone of the Onchocerciasis Control Programme in the Volta Basin, Guillet et al. (1977) noted on the periphery of that zone (in Mali, the Ivory Coast, Togo and Benin) levels of DDT resistance representing coefficients of over 20 in various species of the S. damnosum complex. It should be noted that S. damnosum s.tr. and S. sirbanum are slightly less susceptible than S. soubrense (Table 2).

As none of the rivers from which the resistant strains had been collected had been treated with DDT, the authors concluded that the selective pressure had arisen from the utilization of that insecticide for protecting cotton fields from which it is washed by the rains into the watercourses.

Finally, mention should be made of coefficients of resistance to DDT of the order of 10 to 50 recorded in S. hargreavesi in Mali and Upper Volta by Quélennec & Vervent (1970).

4. CONCLUSIONS - PRIORITY RESEARCH NEEDS

Selection and standardization of one or more methods for determining the susceptibility to insecticides of blackfly larvae; provision of the necessary materials to investigators.

Evaluation of the tests for adults in the field; determination of any correlations between resistance in larvae and the level of susceptibility of adults.

Monitoring of susceptibility to insecticides in the areas where control campaigns are in progress and also in areas that are subjected to intense selective pressure by pesticides used in agriculture.

Investigation of possible cross-resistance between DDT and insecticides intended for use in blackfly control, such as methoxychlor or chlorphoxim, for example.

Provision for biochemical and genetic studies of resistance phenomena. It would be well to re-evaluate by standardized methods the resistance of Simulium ornatum to organophosphorus insecticides in Japan, which is still the sole example in this group of insecticides.

TABLE 1. DIFFERENT METHODS OF EVALUATING THE SUSCEPTIBILITY TO INSECTICIDES OF BLACKFLY LARVAE

Experimenters	Period and mode of exposure	Period and mode of observation	Mortality among controls
Lea & Dalmat 1954 - Guatemala	30 minutes non-aerated water	48 hours running water	High
Muirhead-Thomson 1957 - Liberia	1 hour aerated water	24 hours in same receptacle - aerated water	2-3%
Jamnback 1962, USA Jamnback & West 1970	20 minutes)non-aerated 30 minutes)water	24 hours in running or aerated water	
Ovazza + Valade 1963 - Upper Volta	30 minutes non-aerated water	24 hours in aerated or stagnant water	
Suzuki et al. 1963 Japan	10 minutes in suspension of formulation	24 hours in running water	
Thompson 1975 Ghana	30 minutes non-aerated water	20 hours - aerated water laboratory 20 hours - running water field	9% 4%
WHO 1975 (not satisfactory for Simulium damnosum)	30 minutes non-aerated water	24 hours in same receptacle - aerated water	
Quélennec & Vervent 1970 - Upper Volta	24 hours - non-aerated pre-oxygenated water	No observation; immediate reading	2%
Mouchet et al. 1977 Ivory Coast	3 hours - non-aerated pre-oxygenated water	No observation; immediate reading	2%
Raybould 1966 Tanzania - for neavei complex	30 minutes aerated water	48 hours aerated water	4-8%

TABLE 2. RESISTANCE TO DDT OF SIMULIUM DAMNOSUM S.L. IN WEST AFRICA

Origin of blackflies	LC_{50}	LC_{95}	Coefficient of resistanc at LC_{95} level
Susceptible populations			
4 populations from Ivory Coast	0.045	0.12	
Resistant populations			
Mali - Banifing			
S. damnosum + S. sirbanum	0.098	2.5	21
Mali - Baoule			
S. damnosum + S. sirbanum	0.092	0.62	5.2
Ivory Coast - Marahoué			
S. soubrense	0.44	2.5	21
Togo - Sossoa			
S. damnosum + S. sirbanum	0.029	1.25	10.2
Benin - Kiakiko			
S. damnosum + S. sirbanum	0.033	1.25	10.4

REFERENCES

Anonymous (1975a) Instructions for determining the susceptibility or resistance of adult blackflies, sandflies and biting midges to organochlorine insecticides (WHO mimeogr. document WHO/VBC/75.589)

Anonymous (1975b) Provisional instructions for determining the susceptibility or resistance of blackflies larvae to insecticides (WHO mimeogr. document WHO/VBC/75.591)

Asahina, S. et al. (1966) Insecticide resistance of larvae of Simulium ornatum, Jap. J. Sanit. Zool., 17 (4), 243

Brown, A. W. A. & Pal, R. (1973) Insecticide Resistance in Arthropods, WHO Monograph series, No. 38, 491 pp.

Guillet, P. et al. (1977) DDT resistance in Simulium damnosum in West Africa (WHO mimeogr. document WHO/VBC/77.678)

Jamnback, H. (1962) An electic method of testing the effectiveness of chemicals in killing blackfly larvae, Mosq. News, 22, 384-389

Jamnback, A. & West, A. S. (1970) Decreased susceptibility of blackfly larvae to DDT in New York State and eastern Canada, J. econ. Entomol., 63, 218-221

Lea, A. O. & Dalmat, H. T. (1954) Screening studies of chemicals for larval control of blackflies in Guatemala, J. econ. Entomol., 47, 135-141

Mouchet, J. et al. (1977) Méthodologie pour tester la sensibilité aux insecticides des larves de Simulium damnosum, Cah. ORSTOM, sér. Ent. méd. Parasitol., 15 (1), 55-67

Muirhead-Thomson, R. C. (1957) Laboratory studies on the reactions of Simulium larvae to insecticides, Part I-III, Amer. J. trop. Med. Hyg., 6, 920-934

Ovazza, M. & Valade, M. (1963) Recherches sur la prophylaxie de l'Onchocercose humaine en Afrique de l'Ouest de langue française. II. Essais de larvicides sur le terrain et en laboratoire, Bull. Inst. franc. Afr. noire A., 24, 1215-1234

Quelennec, G. & Vervent, G. (1970) Mesure de la sensibilité aux insecticides des larves de Simulies, Cah. ORSTOM, sér. Ent. méd. Parasitol., 8, 21-44

Raybould, J. N. (1966) A simple laboratory method for testing the susceptibility of larvae of Simulium neavei complex to insecticides, Bull. Wld Hlth Org., 35, 887-892

Suzuki, T., Ito, Y. & Harada, S. (1963) A record of blackfly larvae resistant to DDT in Japan, Jap. J. Exp. Med., 33, 41-46

Thompson, B. H. (1975) Testing the susceptibility of Simulium damnosum (Dipt. Simuliidae) to insecticides in the laboratory and in the field, Bull. Wld Hlth Org., 52, 351-357

Walsh, J. F. (1970) Evidence of reduced susceptibility to DDT in controlling Simulium damnosum on the river Niger, Bull. Wld Hlth Org., 43, 316-318

West, A. S. (1967) The susceptibility of blackflies to DDT including field residues, WHO Inf. Circular on Insect. Resistance, No. 61, p. 14.

= = =