Bull. Org. mond. Santé
Bull. Wld Hlth Org. } 1956, 15, 389-401

THE SIGNIFICANCE OF INSECTICIDE-RESISTANT STRAINS

With Special Reference to Pests of Medical Importance

J. R. BUSVINE, D.Sc.

Reader in Entomology Applied to Hygiene,
London School of Hygiene and Tropical Medicine

SYNOPSIS

The author discusses the meaning of insecticide resistance and the manner in which it should be detected and measured. From some recent data he gives a number of examples of measurements of resistance in mosquitos of different species in various areas. He then proceeds to a speculative discussion on the way in which insecticide resistance arises, whether it can be prevented and overcome, its importance in the past and at present, and finally its future prospects.

The purpose of this paper is not to provide a summary of the scientific work on insecticide resistance; that has been quite adequately reviewed by Metcalf.[25] The intention is to provide a speculative discussion on certain points of practical interest. In addition, some very recent data on resistant strains of mosquitos are put on record.

What is meant by Insecticide Resistance?

For a long time workers in applied entomology have used the term " resistance " to refer to a natural characteristic of a species. In this sense, we could say that *Culex fatigans* is more resistant to DDT than *Anopheles gambiae*. In recent years, however, the word has been widely used to describe strains or races, within species, which are abnormal in being less susceptible to insecticides than the original or natural population of the species. In fact, such strains usually, if not invariably, arise after the extensive use of insecticides and the selective mortality resulting therefrom. For the remainder of the paper, the word will be used in the secondary or special sense.

" Physiological resistance " denotes enhanced capacity to survive a poison.

" Behaviouristic resistance " implies the avoidance of lethal contamination with a poison.

In an analogous way, one must distinguish " natural " behaviouristic resistance, characteristic of the original behaviour of a species (e.g., avoidance of DDT by *A. gambiae*), from " developed " behaviouristic resistance, as shown by strains of *A. albimanus* in certain areas, following intensive use of DDT.[29]

How should Resistance be Detected and Measured?

The first indication of incipient resistance has usually come from field workers, who see that pests are not being killed by treatments which ought to be effective. However, there are several possible causes for failure of insecticides in the field and it is desirable to verify or exclude the possibility of physiological resistance as soon as possible. The proper course is to make comparative measurements of susceptibility levels under as standard conditions as possible. This should also give an indication of the magnitude of the resistance. The test method can be quite simple, but it must rest on sound principles, which will now be considered.

Condition of the insects

The susceptibilities of insects normally vary very much with age, state of nutrition, and temperature. Unless such things are standardized to some extent, any test method will give such variable results that only very gross types of resistance can be demonstrated.

The difficulty is not so serious in cases where insects from treated and untreated localities can be reared together and treated side by side. However, when comparisons are to be made from one year to another or from one country to another, the difficulties of standardization require careful consideration.

A partial solution may be to make relative measurements of susceptibility to two different insecticides. (A pronounced change in the ratio of susceptibility to two compounds may indicate resistance to the one being used in the field.)

In regard to mosquitos, the following points may be noted:

(*a*) *Larvae.* Most experimenters find larvicide tests give very variable results, probably because larvae change their age and physiological condition rapidly. The susceptibility of normal larvae will alter considerably in the course of a single day, especially with the onset of moulting.

(*b*) *Adults.* Male mosquitos are almost always much more susceptible than females, and unfed females are more susceptible than fed ones. The onset of hibernation is likely to affect certain species. Susceptibility to DDT will increase at lower temperatures.

Conditions of the test

Measurements of resistance have been made in many different ways, the two main types of test being those based on dosage and those based

on time of exposure. Measurements based on time of exposure to a residue (or aerosol) suffer from the fact that they are based on the improbable assumption that dosage increases regularly with time of exposure. In methods based on dosage received in a standard treatment, batches of insects are exposed at different levels, to obtain a dosage-mortality curve. Dosage can be applied:

(1) By topical application. This is a satisfactory method, but difficult to do with mosquitos, owing to their fragility. Different species vary in their sensitivity to handling.

(2) By exposure to a standard aerosol at various concentrations. This is difficult to carry out except in a well-equipped laboratory.

(3) By exposure to standard residues. This is usually the most convenient and satisfactory method.

Different methods of measurement may record quite different degrees of resistance for a particular strain. Two examples will illustrate this: (*a*) a DDT-resistant strain of flies from Sardinia was investigated by a topical-application method, which indicated a resistance of $\times 300$ when acetone was used as the DDT solvent, but only $\times 16$ when oil was used; [3] (*b*) a laboratory-selected DDT-resistant strain of *Drosophila melanogaster* was tested by exposure to DDT residues in a treated cage. With a 24-hour exposure, the degree of resistance indicated was $\times 2000$; but with a 6-hour exposure, it was only $\times 6$.[8] These examples show the desirability of adopting standard methods of measuring resistance.

Methods recommended for mosquitos

(*a*) *Adults.* The WHO Expert Committee on Malaria has advocated a method based on that of Busvine & Nash.[6] Full details are given in the fifth report of the Committee.[32]

(*b*) *Larvae.* The adoption of a standard method for measuring resistance of larvae would seem highly desirable. It would be convenient to regularize a method in which DDT in a particular miscible solvent (e.g., alcohol or acetone) is added to water in a standard way. Variations of this type of method have been widely used and have given, for example, the following 24-hour median lethal doses (MLD), in parts per million (p.p.m.), for normal *Aëdes aegypti*: Ginsburg [16] (1947) 0.011; Deonier et al.[9] (1949) 0.003; Pal & Sharma [26] (1952) 0.050; Wharton [31] (1955) 0.008; Elliott [11] (1955) 0.006; Brown & Perry [2] (1956) 0.004.

Examples of measurements of resistant strains of mosquitos

(1) *Measurements on adults by the Busvine method*

(*a*) Anopheles sacharovi *in Greece and Lebanon.* Field observations of insecticide resistance of *A. sacharovi* have been made in Greece since 1951 [24]

and in the Lebanon since 1954.[13] Measurements of resistance were made in 1954 and 1955, as follows:

Area	*Date*	*MLC* (%)*	
		DDT	*dieldrin*
Greece, Agoulinitsa (Lividas [23])	1954	1.2	—
„ Skála (Lividas [23])	1954	2.6	—
„ Skála (Busvine **)	1955	>3	>1
Lebanon, Arida (Busvine **)	1955	1.7	—

* Median lethal concentration
** Unpublished report to WHO, October 1955

Unfortunately no data are available from areas where insecticides have never been used, so that the normal susceptibility of *A. sacharovi* is unknown. The tolerance level at Agoulinitsa was the lowest found by Professor Livadas, but in his opinion there was some indication of incipient resistance even there.

(*b*) Anopheles sundaicus *in Java.* The development of DDT-resistance in *A. sundaicus* was first reported by Crandell [7] in 1954 and was later observed by Chow.[a] Measurements of resistance levels were made in 1955 by Davidson.[b]

Insecticide	*MLC (%)*	
	Semarang (DDT-sprayed area)	*Tjilatjap (unsprayed area)*
DDT	9.0*	0.5
Dieldrin	0.08	0.065
Gamma-BHC	0.0085	0.008
Aldrin	0.4	0.37

* Extrapolated from probit/log concentration graph.

(*c*) Anopheles stephensi *in Saudi Arabia.* Preliminary reports of resistance in *A. stephensi* were made by Dr R. H. Daggy and Dr R. L. Peffly. Measurements of resistance levels were made by Davidson in 1955,[b] both in Saudi Arabia and in London.

Insecticide	*MLC (%)*		
	Al-Haza (DDT-sprayed area)	*Ain-es-Saih (unsprayed area)*	*London (laboratory colony)*
DDT	5.0	<0.5	1.4
Dieldrin	0.17	<0.1	0.12
Gamma-BHC	0.013	<0.05	0.014
Aldrin	0.5	<0.2	0.03

a See note on page 785 of this number of the *Bulletin.*
b Unpublished report to WHO, December 1955

(*d*) Anopheles gambiae *in northern Nigeria.* Elliott & Ramakrishna [12] have recently reported resistance to dieldrin in northern Nigeria in a large trial area sprayed with that insecticide. Their measurements of resistance are compared below with some made by Busvine on *A. gambiae* colonies in London and by Halcrow in Mauritius.[5]

	MLC (%)		
Insecticide	*Birnin-Kebbi, Nigeria (dieldrin sprayed)*	*London (laboratory colony)*	*Mauritus (wild; unsprayed area)*
DDT	1.6	0.95	0.7
Dieldrin	2.0	0.25	0.25
Gamma-BHC	—	0.015	—

(2) *Measurements of resistance in larvae*

The suspensions were prepared by adding DDT in acetone solution to a large quantity of water.

(*a*) Culex fatigans *in Malaya.* Reports of insecticide-resistant strains of *C. fatigans* have been received from India [27] and elsewhere,[19] but precise data are available only on a strain from Malaya.[28]

	24-hour MLD (p.p.m.)	
Insecticide	*Kuala Lumpur (unsprayed)*	*Penang (BHC-treated)*
DDT	0.224	0.245
Gamma-BHC	0.026	0.257
Dieldrin	0.006	0.060

(*b*) Aëdes aegypti *in Trinidad.* DDT-resistance in a strain of *Aëdes aegypti* in Trinidad was reported by Gillette in 1954.[15] The degree of resistance was measured by Brown on a subcolony at the Communicable Disease Center, Savannah, Ga., USA.[2]

	24-hour MLD (p.p.m.)	
Insecticide	*Savannah (laboratory colony)*	*Trinidad colony*
DDT	0.004	2.0

How does Resistance Arise?

If resistant strains develop as a result of selective mortality due to the wide use of an insecticide, the possibility of a strain emerging and the speed with which it will develop depend on three factors:

(1) the number and character of resistant genes in the original population;

(2) the intensity of selection, i.e., the magnitude of the population exposed and the proportion killed;

(3) the number of generations of the treated insect per year.

The first two points require rather careful consideration.

If we think of an insecticide continually killing off the more susceptible members of a population it seems obvious that a more resistant race will result from the " survival of the fittest " (in a rather special sense). However, though we all know that individual insects vary in susceptibility, we also know that much of this variation is due to chance accidents of environment and is not inherited. If all variations were of this type, no amount of selection would raise the level of resistance.

Probably some variability of susceptibility in all insects depends on genetic constitution, though it may be indirect. For example, hereditable variability in size or in resistance to starvation, desiccation, and so forth could influence resistance to insecticides. These characters, however, would be unlikely to vary enormously. Regarding size, for example, it is unlikely that a race of insects more than two or three times larger than normal could be developed by selection. Therefore, natural indirect characters seem unlikely to give rise to very great degrees of resistance.

On the other hand, certain species possess genes promoting specific physiological resistance to particular insecticides. These genes are quite unbeneficial as far as normal existence in the absence of insecticides is concerned. They may even be slightly harmful, which would explain why they are rare. But when a large population containing a few individuals possessing these genes is suddenly exposed to the abnormal selective action of widespread insecticides, the effect is dramatic. The proportion of individuals with resistant genes rises rapidly, and, where multiple genes are present, their combination in the progeny will render the latter even more immune to the insecticide than the parents.

It must be admitted that much of the foregoing argument is somewhat speculative, because very few people have made extensive investigations of the range of variation existing in populations of insects *before* the wide use of insecticides. I think, however, that these theories are reasonable, and, if so, it is clear that happenings in the vast natural populations in the field cannot always be duplicated in the relatively small inbred populations in a laboratory.

With regard to the second factor, intensity of selection, it is clear that if only a small proportion of an insect population is coming into contact with an insecticide, and if the survivors mix and breed with the general mass, the dilution effect will tend to prevent the emergence of a resistant strain.

However, with the modern methods of using insecticides wholesale, it has been possible to reach a high proportion of insect populations over considerable areas. For example, if all the villages in a certain district are sprayed with DDT, it is likely that most of the flies and mosquitos which habitually infest human dwellings will be affected. Possibly this is more likely to occur with insects of medical importance than with agricultural pests, which would explain the more frequent occurrence of cases of resistance among the former.

Can Resistance be Prevented?

One obvious way to avoid resistance is not to use the insecticide concerned. This is not quite so pointless as it seems, for I have heard it reasonably suggested that in countries where typhus is an ever-present threat, DDT should be saved for epidemics and not used as a general hygiene measure, short of complete eradication of lice. Intermittent use, it was said, would tend to build up resistance among lice and thus lessen the value of DDT for preventing a typhus epidemic.

When the regular use of an insecticide is necessary to control endemic disease (e.g., in antimalarial work), it may be desirable to restrict the attack to the adult stage. It has been claimed that the simultaneous use of an insecticide against larvae and adults is prone to develop resistance more quickly than its use against adults only. This seems quite possible, in view of the greater proportion of the insect population exposed to selective action, especially in the wholesale application by aerial spraying.

An opposite policy has also been advocated, sometimes supported by rather obscure logic. It is suggested that resistance develops from inadequate dosing and that heavy applications should be used to prevent it. However, this is only certain to be true where the insecticide produces complete extermination. Otherwise it is difficult to see why a higher mortality should not produce more-rapid selection for resistance.

Can Resistance be Overcome?

Increasing the dosage

When an insect can develop a specific physiological resistance—such as that of the housefly to certain kinds of chlorinated hydrocarbons—the level of tolerance may be raised several hundred times. Obviously it is not feasible to attack them by increasing the dosage, for one is not able to contaminate them with enough poison to compensate for the increased resistance.

However, it is quite possible that direct and specific resistance mechanisms do not exist in all types of insect for all types of poison. Where a

specific resistance does not exist, it is still possible for an insect to develop resistance through indirect factors (e.g., enhanced " vigour ") but, as already explained, one would not expect this form of resistance to reach very high levels. In such a situation it may be possible to attain permanent control by increasing the dosage. (Possibly this may have occurred in the Tennessee Valley, where the dose necessary to kill *A. quadrimaculatus* larvae has apparently increased in recent years, but satisfactory control is still obtained.[14]

Increasing the dosage is not always as simple as it may appear. For example, if we consider residual wall-deposits of insecticide, it may be easy to double the deposit, but this may not substantially affect the contamination of the mosquito which alights on it. Hadaway & Barlow[18] found no significant increase in the kill of mosquitos at rates of deposit above 3 mg of DDT per square foot. (The heavy deposits necessary in the field are to give longer action, not greater immediate toxicity.)

More effective than increasing the rates of application of residual insecticides may be improvements in formulation, times or places of spraying, etc.

Changing the insecticide

When pronounced forms of resistance have developed, one can change to another type of compound in the hope that the pest will not be able to become resistant to that also. However, apart from the question of whether that will eventually happen or not, the choice of really suitable alternative insecticides is not as great as might appear.

Many of them can be grouped into classes, and resistance developed to one member of the group automatically provides a defence against the others. Three examples of such group resistance have been observed:

(*a*) Resistance to DDT and its analogues (methoxychlor, DDD, etc): *Musca domestica, Pediculus humanus.*

(*b*) Resistance to gamma-BHC, chlordane, heptachlor, dieldrin, aldrin, endrin, isodrin, and toxaphene: *M. domestica, Blattella germanica, Culex fatigans, C. tarsalis, Boophilus decoloratus.*

(*c*) Resistance to various organo-phosphorus insecticides: *Tetranychus telarius, M. domestica.*

Supposing it is feasible to use two or three different types of insecticide, it may be possible to defeat resistance by changing from one to another at suitable intervals. This is only likely to succeed if the resistance to the first insecticide declines fairly rapidly when it is discontinued or superseded. Incidentally, we must remember that residual insecticides such as DDT do not necessarily cease to have an effect as soon as their use is discontinued, but decline in activity gradually. It is true that if the selective influence of an insecticidal treatment is removed, many laboratory colonies decline in

resistance in the course of a year or so. The decline is presumably due to the fact that the genes responsible for resistance are either linked with other unfavourable genes or else themselves have additional effects which are unfavourable. For example, a number of DDT-resistant strains of housefly have been found to have life-cycles which are slightly longer than normal. This is not a serious disadvantage, but it might well be the kind of factor responsible for the slow elimination of resistant members of the population in the absence of insecticides. Since the decline in resistance appears to be a slow process, there does not seem to be much hope of alternating between different kinds of insecticides.

An alternative suggestion has been to use mixtures of different kinds of insecticide at the same time. The possibilities of this depend on whether susceptibility to the two substances is positively correlated, quite independent, or negatively correlated. In the first place the mixture will achieve nothing, or at the most will act like a slightly increased dose of the strongest insecticide. In the second case there will also be little additional effect. If, however, it happens that the order of susceptibility to compound A is the reverse of that to compound B, then the mixture will have distinct possibilities. For the resistant survivors from compound A will be exterminated by compound B. However, the existence of two compounds of this type has yet to be demonstrated.

What was the Occurrence of Resistance in the Past?

It is well known that resistant strains are not an entirely recent occurrence. The earliest examples are the Californian scale insects, which developed races resistant to lime sulfur and to hydrocyanic acid; these observations were made some fifty years ago. Nevertheless, despite the fairly extensive use of insecticides since then, the number of instances of resistance prior to 1940 is quite small, not more than two or three, if one excludes doubtful cases and artificially induced resistance in laboratory strains.

In the last decade, however, there has been a considerable increase in the number of cases of resistance. Many of these are concerned with synthetic chlorinated-hydrocarbon insecticides. It is not clear whether this is because these compounds are more liable to permit resistance mechanisms to develop in insects or whether their very extensive use has resulted in the present situation. Possibly each factor is partly responsible.

What is the Present Situation regarding Resistance?

It is difficult to make an exact statement on the occurrence of resistant strains at the present time, because the information available varies so much in detail and precision. At one extreme, we have the almost universal

example of the housefly, which has been the subject of numerous scientific investigations, and at the other are verbal reports, based on general impressions, which have appeared in print with the reference " personal communication ".

The following list is slightly conservative, since it includes only cases supported by published data or by unpublished reports submitted to WHO:

Resistant Strains of Insects of Medical Importance

(1) The housefly (*Musca domestica, Musca vicina*)	DDT	(1947) [30]	World-wide
	chlordane etc.*	(1949) [25]	World-wide
(2) *Culex fatigans*	DDT	(1953) [19]	Réunion
	BHC	(1954) [27]	India
	BHC etc.	(1955) [28]	Malaya
(3) Salt-marsh-breeding culicines			
Aëdes taeniorhynchus, *Aëdes sollicitans*	DDT	(1950) [10]	Florida
Aëdes nigromaculus	DDT	(1950) [1]	California
Culex tarsalis	chlordane etc.*	(1951) [17]	California
(4) *Anopheles quadrimaculatus*	DDT	(1952) [14]	Tennessee
(5) *Anopheles sacharovi*	DDT	(1953) [24]	Greece
	chlordane etc.*	(1954) [23]	Greece
(6) *Anopheles sundaicus*	DDT	(1954) [7]	Java
(7) *Anopheles gambiae*	dieldrin	(1955) [12]	Nigeria
(8) *Anopheles stephensi*	DDT	(1955) **	Saudi Arabia
(9) *Aëdes aegypti*	DDT	(1954) [15]	Trinidad
(10) *Pediculus humanus*	DDT	(1952) [20]	Korea
		(1953) [4]	Egypt
(11) *Cimex lectularius*	DDT	(1948) [21]	Hawaii
		(1953) [22]	Israel

* Implies resistance to some and probably all of the compounds in group (*b*) mentioned earlier (see page 396).

** Davidson, G., unpublished report to WHO, December 1955

Measurements of resistance of *A. sacharovi* in Greece suggest that quite a small level of resistance ($\times 3$ to $\times 5$) may result in the survival of these mosquitos. It is, of course, easy to increase the wall-deposits by such a factor (though perhaps not economically possible); but this may not alter the dosage picked up by the mosquito.

From what has been said, then, it would seem that considerable risks of immunity to insecticides exist in cases where an insect pest has a naturally low susceptibility and the margin of safety is already small.

In attempting to predict the future importance of resistance, one is handicapped by the fragmentary nature of the information about present examples. Therefore it is very desirable to establish the following facts for all cases of suspected resistance:

(1) The level of resistance should be measured and its geographical extent determined.

(2) The type of resistance should be investigated to distinguish between specific forms (probably depending on some biochemical mechanism) and general immunity (possibly due to indirect causes, such as " vigour ", thicker cuticle, etc.).

(3) If physiological resistance has been excluded, the possible existence of a race showing behaviouristic change should be investigated.

What are the Future Prospects of Resistance?

It is evident that the ability to develop resistance differs considerably from one species of insect to another and also depends on the insecticide used. The rather rapid, ubiquitous, and extremely high levels of resistance shown by the housefly exposed to chlorinated-hydrocarbon insecticides is clearly exceptional. Personally, I have always been impressed by the anomaly of this resistance and have been inclined, perhaps wrongly, to underestimate the gravity of the threat of resistance in other species. However, while it still seems to be true that the enormously high, specific type of resistance is rare, it must be emphasized that quite low levels of resistance may have serious practical consequences.

The practical importance of enhanced resistance depends on the margin of effectiveness possible with the insecticide treatment used. Now, the control of mosquitos (and perhaps other insects) by residual spraying of walls does not appear to have a large margin of effectiveness. Quite apart from resistant races, there have always been some species of low susceptibility to DDT, such as *C. fatigans*; and others, like *A. gambiae*, which were easily irritated and often escaped with a non-lethal dose.

ACKNOWLEDGEMENT

I am indebted to Mr George Davidson for permission to quote his data on resistant strains in Java and Saudi Arabia, which have not previously been published.

RÉSUMÉ

La résistance physiologique de certains insectes à des substances chimiques se trahit dans la pratique par l'échec du traitement au moyen des insecticides. Pour s'assurer qu'il s'agit bien d'une résistance physiologique — et non d'une baisse de qualité de l'insecticide ou de toute autre cause — divers tests ont été proposés. Ces épreuves peuvent être simples, mais doivent être effectuées dans des conditions aussi uniformes que possible, afin que leurs résultats soient comparables. Or, la résistance d'une population peut varier selon

l'âge des moustiques, leur état nutritionnel et la température; la sensibilité des larves peut même varier au cours de la même journée, en particulier au début de la mue; les mâles adultes sont plus sensibles que les femelles et les femelles à jeun plus sensibles que les femelles gorgées; la sensibilité au DDT augmente aux basses températures. Il est donc nécessaire d'uniformiser ces conditions dans la mesure du possible avant de soumettre les insectes aux tests. Les résultats peuvent varier considérablement selon la méthode employée. La plus pratique est celle qui consiste à exposer les insectes adultes à des quantités résiduelles d'insecticide standardisées.

La méthode de Busvine & Nash a été recommandée par le Comité OMS d'experts du Paludisme, pour l'évaluation de la résistance des moustiques adultes. L'auteur mentionne quelques résultats obtenus par cette méthode avec divers insectes dans plusieurs pays.

Si l'on considère la résistance comme la conséquence d'un phénomène de mortalité sélective due à l'emploi intensif des insecticides, l'apparition d'une souche résistante et sa vitesse de développement dépendra du nombre et des caractères des gènes responsables de la résistance dans la population initiale, de la grandeur de la population exposée, de la proportion détruite par l'insecticide et du nombre de générations annuelles de l'insecte soumis à l'action de l'insecticide. Il est probable que, dans certains cas, la résistance aux insecticides dépend indirectement de la constitution génétique, et qu'elle est liée à des caractères tels que la taille, la résistance à la dessiccation ou à la sous-alimentation. Lorsqu'une population nombreuse, comprenant quelques individus possédant les gènes « résistants » est mise en présence de l'insecticide, l'effet est saisissant: la proportion d'individus résistants croît rapidement, et il se peut que les combinaisons géniques entre individus résistants donnent naissance à des souches plus résistantes encore que les souches initiales. Ces considérations sont en partie théoriques, car les données manquent sur la variabilité de la résistance au sein de populations de moustiques, avant l'emploi des insecticides. Il est évident d'autre part que les expériences de laboratoire ne reflètent pas exactement les phénomènes qui se déroulent à l'échelle de la nature.

Il est difficile de définir la portée actuelle du problème de la résistance. Les données sûres sont peu nombreuses, si l'on excepte les observations concordantes faites dans le monde entier sur la mouche domestique, dont la résistance généralisée paraît un phénomène exceptionnel parmi les arthropodes. Il faut souligner toutefois que même des degrés de résistance faible peuvent avoir des conséquences pratiques, particulièrement dans les cas où l'insecte est normalement peu sensible à l'insecticide et lorsque la marge entre sensibilité et résistance est étroite.

L'insuffisance et l'imprécision des informations rendent également les prévisions hasardeuses. Chaque fois que l'on soupçonne l'apparition d'une résistance chez une espèce, il y a lieu d'établir, en particulier, son intensité, son extension géographique, et sa nature (résistance spécifique ou résistance biologique générale liée à d'autres caractères).

REFERENCES

1. Bohart, R. M. & Murray, W. (1950) In: *Proceedings of the Eighteenth Conference of the Californian Mosquito Control Association*, Turlock, Calif., p. 20
2. Brown, A. W. A. & Perry, A. S. (1956) *Nature (Lond.)*, **178**, 368
3. Busvine, J. R. (1951) *Nature (Lond.)*, **168**, 193
4. Busvine, J. R. (1953) *Nature (Lond.)*, **171**, 118
5. Busvine, J. R. (1956) *Nature (Lond.)*, **177**, 533
6. Busvine, J. R. & Nash, R. (1953) *Bull. ent. Res.*, **44**, 371
7. Crandell, H. A. (1954) *Mosquito News*, **14**, 194
8. Crow, J. (1954) *J. econ. Ent.*, **47**, 393
9. Deonier, C. C. et al. (1949) *Mosquito News*, **9**, 150
10. Deonier, C. C. et al. (1950) *J. econ. Ent.*, **43**, 506

11. Elliott, R. (1955) *Trans. roy. Soc. trop. Med. Hyg.*, **49**, 528
12. Elliott, R. & Ramakrishna, V. (1956) *Nature (Lond.)*, **177**, 532
13. Garrett-Jones, C. & Gramiccia, G. (1954) *Bull. Wld Hlth Org.*, **11**, 865
14. Gartrell, F. E. & Ludvik, G. (1954) *Amer. J. trop. Med.*, **3**, 817
15. Gillette, H. P. S. (1954) In: *Annual report of the Malaria Division, Health Department, Trinidad and Tobago*, Port of Spain
16. Ginsburg, J. M. (1947) *Science*, **105**, 233
17. Gjullin, C. M. & Peters, R. (1952) *Mosquito News*, **12**, 1
18. Hadaway, A. B. & Barlow, F. (1951) *Bull. ent. Res.*, **41**, 603
19. Hamon, J. (1953) *Bull. Soc. Path. exot.*, **46**, 454
20. Hurlbut, H. S. et al. (1952) *Science*, **115**, 11
21. Johnson, M. S. & Hill, A. (1948) *Med. News Letter Bur. Med. Surg. (Wash.)*, **12**, 27
22. Levinson, Z. H. (1953) *Riv. Parassit.*, **13**, 57
23. Livadas, G. A. (1955) *Mosquito News*, **15**, 67
24. Livadas, G. A. & Georgopoulos, G. D. (1953) *Bull. Wld Hlth Org.*, **8**, 497
25. Metcalf, R. L. (1955) *Physiol. Rev.*, **35**, 197
26. Pal, R. & Sharma, M. (1952) *Indian J. Malar.*, **6**, 275
27. Rajagopalan, N. et al. (1954) *Bull. nat. Soc. India Malar. & mosquito-borne Dis.*, **2**, 211
28. Reid, J. (1955) *Bull. Wld Hlth Org.*, **12**, 705
29. Trapido, H. (1952) *Amer. J. trop. Med.*, **1**, 853
30. Weismann, R. (1947) *Mitt. schweiz. ent. Ges.*, **20**, 484
31. Wharton, R. H. (1955) *Bull. ent. Res.*, **46**, 301
32. World Health Organization, Expert Committee on Malaria (1954) *Wld Hlth Org. techn. Rep. Ser.*, **80**