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Observations on Doubly Resistant *Anopheles aconitus* Dönitz in Java, Indonesia, and on its Amenability to Treatment with Malathion

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The development of resistance by a single vector population to the two main insecticides (DDT and dieldrin) used in malaria eradication programmes is as yet a comparatively rare event, but one of a potentially serious nature when it does occur.

This paper describes the background of one such instance—double resistance by Anopheles aconitus *in Java—and gives the results of observations made on this mosquito population, including its reaction to sprayed structures.*

Judged on the basis of the mortality produced and of the reduction in biting in sprayed structures, DDT was found to have little effect on A. aconitus. *Malathion, on the other hand, gave fairly satisfactory results under the conditions prevailing in the experimental area.*

In March 1962, *Anopheles aconitus*, one of the main malaria vectors in Java, suddenly appeared in high densities in Jogjakarta Zone, Central Java (Fig. 1). Susceptibility testing showed the species to be resistant to both DDT and dieldrin,[4] exposure to 4% DDT for one hour producing 27% mortality, and exposure to 4% dieldrin for one hour producing 54% mortality in freshly fed females.

The spraying history in the area of Jogjakarta Zone which gave the above figures was as follows: 1957, one cycle of dieldrin at 0.5 g/m²; 1958 and 1959, two cycles of DDT at 2 g/m²; 1960, 1961 and 1962, four cycles of DDT at 1 g/m².

Investigations have shown that in Jogjakarta, as well as in other zones of Central Java, insecticides have been used in the rice-fields to control agricultural pests (DDT since 1952; dieldrin and other cyclodiene derivatives since 1958). It seems that this agricultural use of insecticides is at least partly responsible for the development of insecticide-resistance in the anopheline population.

Further testing showed that the double resistance was widespread in the Jogjakarta Zone. As the neighbouring zone to the east (Klaten) was as yet unsprayed, it was decided to carry out susceptibility tests in this zone in an attempt to determine how far the double resistance extended into the unsprayed area. Owing to the patchy distribution of the *A. aconitus* population, it was possible to carry out tests at three points only in the unsprayed region. The results of these tests are shown in Fig. 2. Unfortunately the interpretation of these figures has been complicated by the use of DDT and of dieldrin analogues for agricultural purposes both in Jogjakarta and in Klaten.

After detection of double resistance, experimental hut observations were undertaken in area A shown in Fig. 2 with a view to determining the effect of DDT on those *A. aconitus* entering sprayed structures in this area, and also to compare this with the effect of malathion, both alone and as a mixture with HCH.

MATERIALS AND METHODS

Initially experimental huts were built which were of the type intended for use with human bait. It soon became apparent, however, that *A. aconitus* were not attracted in sufficient numbers to human bait in these huts despite certain modifications. Accordingly it was decided to use cattle as bait. For this purpose existing cattle-sheds were adapted by enclosing them completely with bamboo walls

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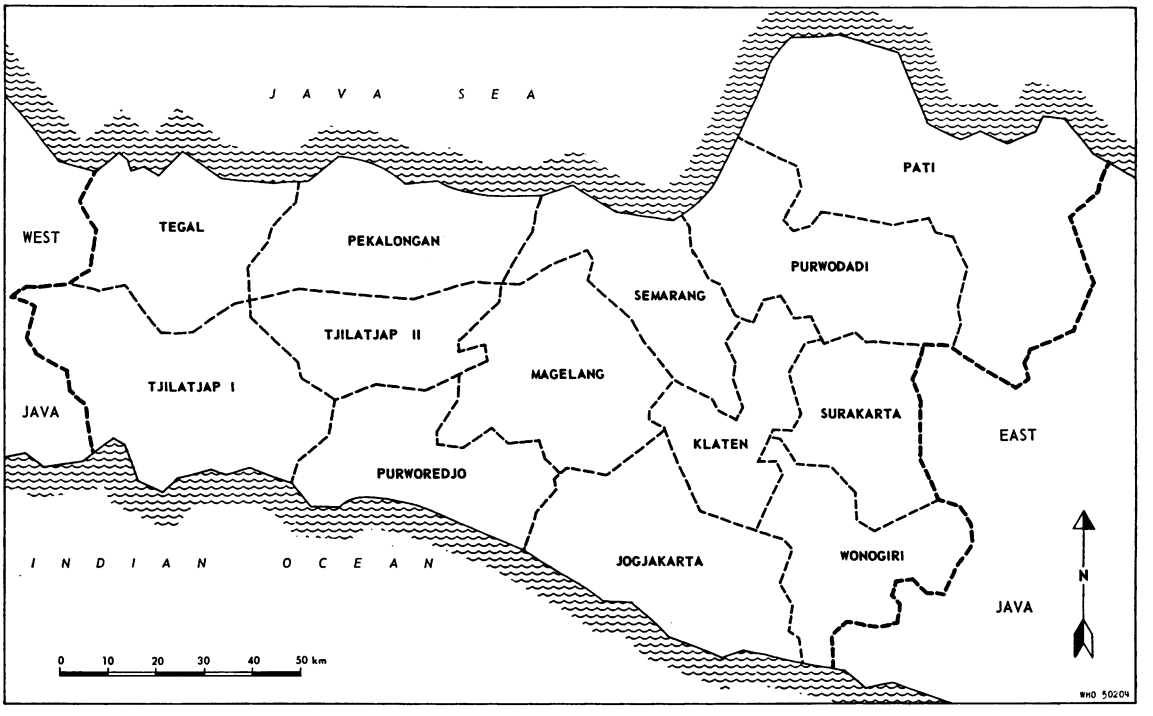
[4] Soerono, M., Davidson, G. & Muir, D. A. (1965) *Bull. Wld Hlth Org.*, 32, 161-168.

FIG. 1
MALARIA ERADICATION ZONES IN PROVINCE OF CENTRAL JAVA



covered with a lining of finely woven bamboo matting. The roofs were of thatch.

To enable a partial floor collection to be made, one-quarter of the floor area in each cattle-shed was covered with whitewashed matting and the cattle were prevented from trampling over this portion. Correction was subsequently made by multiplying the floor catch by four. Entry slots were provided by turning in the lower part of the walls. The internal openings of the slots were 2 cm wide and were horizontal. Two window-traps were used with each cattle-shed, and four cattle-sheds were adapted in this way.

Initially, three of the cattle-sheds contained zebu cattle (*Bos indicus*) and one contained water buffaloes (*Bos bubalus*). During preliminary observations it soon became apparent that much higher catches were being taken with the latter. The average window-trap catch per animal over a five-day period was: water buffalo, 586; zebu cattle, 35.

It was therefore decided that all the experimental cattle-sheds should contain water buffaloes.

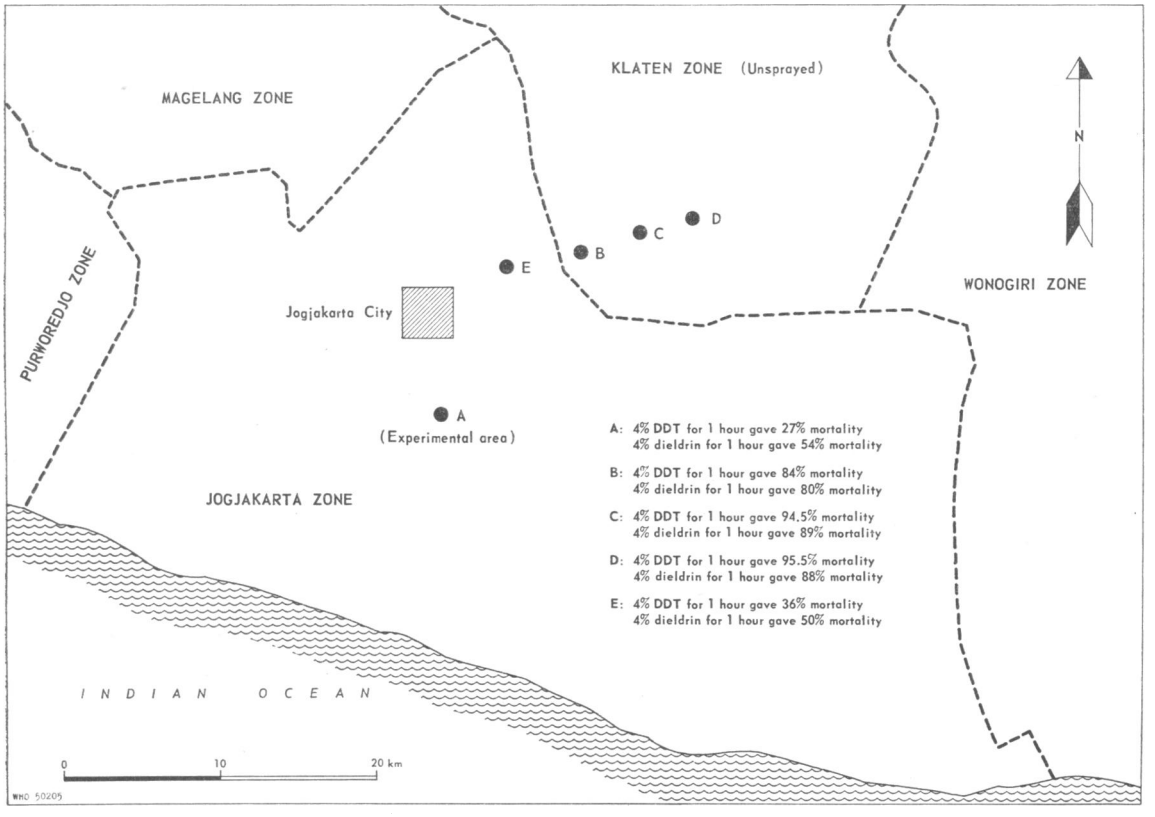
The cattle-sheds were sprayed on 23 March 1963, as follows:

Shed No.	*Insecticide*
1	HCH (0.5 g/m²) + malathion (1 g/m²)
2	DDT (2 g/m²)
3	Malathion (2 g/m²)
4	Control, unsprayed

All three insecticides were applied on interior walls and roofs in the form of water-dispersible powder. The air temperatures and relative humidity ranged between 23°C and 30°C and 65% and 95% respectively during the observation period.

The routine of observation was as follows. Window-traps were removed and replaced between 06.30 hours and 07.30 hours. In the laboratory the contents of each trap were removed and recorded. Live mosquitos were held for observation in paper cups which were kept inside large earthenware pots for approximately 10 hours. The numbers dying during this period were recorded, and the survivors exposed to 4% DDT for one hour as a check on any

FIG. 2
RESULTS OF SUSCEPTIBILITY TESTING FOR DOUBLE RESISTANCE IN *A. ACONITUS*



selective effect of the sprayed cattle-sheds for DDT-resistance. After exposure, the anophelines tested were held for 24 hours in paper cups and the mortalities estimated at the end of this period. The survivors from the HCH/malathion shed were extremely few in number and were not tested for the selection of dieldrin-resistance since the main interest in the present investigations was in DDT-resistance. It may be noted in passing that results from a similar insecticide trial in an area of dieldrin-resistant *A. sundaicus* conducted on the south coast of Java indicate that this mixture has no selective effect on a partially dieldrin-resistant population.[1]

Floor collections were carried out in the cattle-sheds twice daily, at 08.00 hours and 17.00 hours.

Observations were continuous. Window-traps were numbered and used only with the appropriate shed. The traps were washed at least twice a week with detergent, and every effort was made to avoid contamination in other ways.

Mortalities produced among those anophelines entering the cattle-sheds are presented as follows:

$$\% \text{ Mortality} = \frac{\left(\begin{array}{c}\text{No. dead}\\ \text{on floor}\end{array}\right)+\left(\begin{array}{c}\text{No. dead in}\\ \text{window-trap}\end{array}\right)+\left(\begin{array}{c}\text{No. dying in}\\ \text{observation}\\ \text{period}\end{array}\right)}{\text{Total catch}} \times 100.$$

RESULTS

For the pre-spray period (15-23 March 1963), mortalities of mosquitos from the unsprayed cattlesheds were as shown in Table 1. The comparatively high mortalities in shed 3 were probably caused by crowding in the window-traps. Mortalities on subsequent exposure of survivors to 4% DDT-

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TABLE 1
PRE-SPRAY MORTALITIES

Unsprayed shed No.	Percentage mortality [a]		Percentage of catch taken in window-trap
	Total [b]	Freshly fed [c]	
1	1 (144)	0 (110)	100
2	2 (314)	1 (106)	100
3	15 (2 708)	7 (342)	100
4	4 (71)	0 (12)	100

[a] Figures in parentheses refer to numbers of mosquitos.
[b] Females in various stages of gonotrophic cycle.
[c] Freshly fed females only.

TABLE 2
MORTALITY ON SUBSEQUENT EXPOSURE OF SURVIVING MOSQUITOS TO 4% DDT-IMPREGNATED PAPERS FOR ONE HOUR (BEFORE SPRAY)

Unsprayed shed No.	Corrected percentage mortality [a]	
	Total [b]	Freshly fed [c]
1 (Control)	13 (143)	6 (110)
2	45 (268)	24 (105)
3	48 (2 014)	22 (311)
4	45 (68)	2 (12)
2 + 3 + 4	47 (2 350)	22 (428)

[a] Figures in parentheses refer to numbers of mosquitos.
[b] Females in various stages of gonotrophic cycle.
[c] Freshly fed females only.

TABLE 3
COMPARATIVE CATCHES BEFORE AND AFTER INSECTICIDE SPRAYING OF CATTLE-SHEDS

Shed No.	Catch during the two days before spraying	Catch during the two days after spraying	Percentage of pre-spray catch
1 (HCH/Malathion)	111	1	1
2 (DDT)	266	51	19
3 (Malathion)	363	28	8
4 (Control)	48	151	315

impregnated papers for one hour are given in Table 2.

Immediately after the insecticide spraying the catches from the sprayed cattle-sheds fell well below the pre-spray figures (Table 3). The catch from the control cattle-shed increased, however, suggesting a repellency from the sprayed structures and a deviation to the unsprayed shed.

The mortalities resulting during the first 16 weeks after spraying are presented in Table 4, from which it will be seen that the control catch was satisfactory for the first 12 weeks but fell off in the last two fortnightly periods, and that:

(*a*) a high kill was maintained by the HCH/malathion mixture for six weeks and the catch declined after eight weeks;

(*b*) in the DDT-sprayed shed mortality declined rapidly after the first fortnight and thereafter was practically nil; and

(*c*) malathion alone gave practically complete kills for eight weeks, and mortality never fell below 84% during the remainder of the observation period.

The mortalities produced on subsequent exposure of survivors to 4% DDT for one hour are shown in Table 5 as total figures for the 16-week post-spray period. Unfortunately the numbers available for testing from cattle-sheds 1 and 3 were too small for any firm conclusions to be drawn. The figures from the DDT-sprayed shed show a marked reduction in mortality when compared to the other sprayed sheds and corresponding pre-spray figures, suggesting a pronounced selective effect by the DDT-sprayed structure (operating mainly in the first month, since mortalities produced after this were extremely low).

DISCUSSION

Cattle-shed No. 1 (HCH 0.5 g/m²+malathion 1 g/m²)

As can be seen from Table 4, the numbers of *A. aconitus* taken in this cattle-shed were never high and fell to a very low level after eight weeks. Before the fall in numbers occurred, however, the mortality had fallen to 33% among freshly fed females after remaining at over 90% for the first six weeks.

Cattle-shed No. 2 (DDT 2 g/m²)

Here the mortalities produced were never high, reaching 24% of total females and 15% of freshly fed females in the first fortnight after spray. Thereafter the mortalities recorded never exceeded 6%, and for six weeks no mortality was recorded at all.

TABLE 4

CORRECTED POST-SPRAY MORTALITIES AND PERCENTAGES TAKEN IN WINDOW-TRAPS [a]

Shed		1-2 weeks	3-4 weeks	5-6 weeks	7-8 weeks	9-10 weeks	11-12 weeks	13-14 weeks	15-16 weeks
1 (HCH/malathion)	Total	100 (29)	100 (39)	89 (50)	24 (21)	60 (5)	29 (7)	—	—
	Freshly fed	100 (25)	100 (33)	93 (31)	33 (15)	60 (5)	29 (7)	—	—
	% in window-traps	17	18	76	100	100	100	—	—
2 (DDT)	Total	24 (221)	6 (161)	0 (87)	0 (37)	0 (9)	3.3 (61)	6 (17)	—
	Freshly fed	15 (115)	5 (115)	0 (60)	0 (31)	0 (9)	1.7 (58)	0 (10)	—
	% in window-traps	100	100	100	100	100	100	100	—
3 (Malathion)	Total	100 (256)	100 (132)	99 (76)	99 (93)	91 (33)	92 (84)	85 (26)	93 (56)
	Freshly fed	100 (161)	100 (85)	98 (57)	99 (80)	97 (31)	91 (80)	84 (25)	92 (50)
	% in window-traps	61	64	58	96	100	81	100	100
4 (Control)	Total	4.5 (965)	3 (288)	10 (100)	5 (56)	2 (44)	2 (45)	0 (2)	0 (2)
	Freshly fed	0.7 (441)	0.6 (171)	3 (35)	3 (38)	0 (30)	0 (38)	0 (2)	0 (2)
	% in window-traps	100	100	100	100	100	100	100	100

[a] Figures in parentheses refer to numbers of mosquitos.

Cattle-shed No. 3 (malathion 2 g/m²)

For the first two months there was practically complete mortality recorded from this cattle-shed, there being only two survivors (in the second month) from a total of 557 *A. aconitus* females. In the third month the lowest mortality recorded was 91%. In the fourth month the lowest mortality recorded was 84%. The results indicate that under the climatic conditions prevailing in this part of Jogjakarta Zone, on a surface of split and woven bamboo and at a dosage of 2 g/m² malathion was effective for at least four months. This is in agreement with the findings of insecticide trials carried out in an *A. sundaicus* area of the coast of Java (report in preparation).

TABLE 5

MORTALITY ON POST-SPRAY EXPOSURE OF SURVIVING MOSQUITOS TO 4% DDT FOR ONE HOUR

Shed No.	Corrected percentage mortality [a]	
	Total [b]	Freshly fed [c]
1 (HCH/malathion)	24 (21)	36 (11)
2 (DDT)	6 (520)	12 (345)
3 (Malathion)	16 (31)	28 (15)
4 (Control)	12 (1 342)	3 (635)

[a] Figures in parentheses refer to the number of mosquitos.
[b] Females in various stages of gonotrophic cycle.
[c] Freshly fed females only.

Biting, together with escape to the window-trap, increased markedly in the malathion shed after the first six weeks and would appear to indicate a falling off of any post-spray fumigant or particulate effect. Since, at the same time, there was a sharp drop in mortality in the HCH/malathion shed, where one would expect a similar (or enhanced) fumigant/particulate action, it appears likely that the continued high kills recorded from the malathion shed are due mainly to contact with sprayed surfaces rather than to continued fumigant/particulate action on the mosquitos after their escape to the window-trap.

General

As already mentioned, there was an indication of repellency from the sprayed structures (Table 3). This was supported by the total catches recorded for the post-spray observation period. The figures were as follows:

Shed No.	*Total catch*
1 (HCH/malathion)	151
2 (DDT)	594
3 (Malathion)	756
4 (Control)	1 503

The amount of biting in the sprayed structures may be judged roughly by comparing the proportions of unfed females recovered from each cattle-shed (Table 6). It will be seen that the percentage of unfed females taken from the control shed after spraying is very near the pre-spray figure (6.8% and 6.2% respectively). The figures for sheds 1 and 3 show a much higher percentage of unfed females, indicating the probability of reduction in the proportion biting among unfed females entering these sheds. In shed 2 (DDT) there was a proportion of unfed females which was slightly lower than even the control and pre-spray figures. This was also the case in the insecticide trials in the *A. sundaicus* area mentioned above (in so far as the DDT-sprayed experimental huts were concerned). It appears probable, therefore, that a small proportion of the unfed females which, after entering the shed would normally rest and leave again in an unfed state, were so stimulated by contact with the DDT that they took a blood-meal. Alternatively it is possible that this small proportion may be repelled before entry, or both processes may play a part.

In so far as gravid females are concerned, the highest proportion from among the sprayed sheds was produced by the DDT shed and the lowest proportion by the malathion shed. It is possible, however, that some females may enter the sheds when they are already at an advanced stage of the gonotrophic cycle. The apparent slight change in behaviour of some fed females in the control hut after spraying, which resulted in a greater egress of freshly fed females than before spraying, may possibly be related to deviation to the control shed of females deterred from entering the sprayed sheds.

Another noticeable feature is the fact that, whereas before spraying the mortalities on exposure to 4% DDT papers were higher in the " total " group than in the " freshly fed " group, after spraying the position is reversed, higher mortalities being produced in the latter. This could be due to the following factors:

(*a*) greater comparative selection of groups other than freshly fed (i.e., mainly unfed) within the cattle-sheds;

(*b*) comparatively greater contact with insecticides in the sheds by the freshly fed group, this rendering them more liable to succumb to subsequent exposure;

(*c*) a combination of the above.

The process mentioned under (*a*) above would imply a greater percentage mortality in the " total " than in the " freshly fed " categories produced by the sprayed cattle-sheds. There is, however, no evidence of this in the figures for sheds 1 and 3. In shed 2 there is a slightly higher mortality in the " total " than in the " freshly fed " group, but only for the first fortnight, there being little difference thereafter. Besides this, pronounced selection for DDT-resistance would not be expected from sheds 1 and 3. It seems most probable, therefore, that the comparatively greater mortalities produced among the " freshly fed " on subsequent exposure to DDT is due mainly (at least in sheds 1 and 3) to their having previously picked up comparatively higher doses of insecticides.

Since the above observations were limited to three months (owing to a seasonal drop in *A. aconitus* density) further trials have been initiated to determine whether malathion will remain effective for a longer period. Lower concentrations of malathion were included also in these new trials. The results will be presented at a later date.

TABLE 6

PROPORTIONS (%) OF THE VARIOUS GONOTROPHIC CATEGORIES RECOVERED FROM EACH CATTLE-SHED

Mosquitos	Before spraying	After spraying			
	All sheds	Shed 1 (HCH/malathion)	Shed 2 (DDT)	Shed 3 (Malathion)	Shed 4 (Control)
Unfed	6.2	11.3	4.9	15.7	6.8
Freshly fed	17.6	79.5	67.0	76.0	50.5
Gravid	76.2	9.2	28.1	8.3	42.7

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RÉSUMÉ

L'apparition d'une double résistance au DDT et à la dieldrine ayant été constatée chez *Anopheles aconitus* en mars 1962, dans le secteur de Jogjakarta (Java central, Indonésie), des recherches ont été entreprises dans trois étables traitées et une étable témoin afin de déterminer l'effet du DDT à la dose de 2 g/m² sur *A. aconitus* et de le comparer à celui du malathion (2 g/m²) et à celui d'un mélange HCH/malathion (0,5 g HCH + 1 g malathion par m²).

La mortalité observée avec le mélange HCH/malathion, forte pendant les six premières semaines, a diminué rapidement par la suite; au bout de huit semaines, le nombre des moustiques trouvés dans l'étable traitée était trop faible pour donner un résultat significatif. Dans l'étable traitée au DDT, la mortalité fut extrêmement faible dès le début et pratiquement nulle après le premier mois. Avec le malathion, les mortalités enregistrées atteignirent près de 100% pendant les deux premiers mois; pendant le troisième mois, la plus faible valeur obtenue fut 91%.

Une réduction d'activité des moustiques, mesurée par la proportion de femelles non gorgées, put être constatée avec le mélange HCH/malathion, comme avec le malathion employé seul. En revanche, il n'y eut apparemment aucune réduction d'activité chez les moustiques femelles pénétrant dans l'étable traitée au DDT.

D'après les observations faites sur les insectes recueillis dans l'étable traitée au DDT, il a semblé que ce produit exerçait sur *A. aconitus* un effet de sélection dans le sens de la résistance mais il ne faut pas exclure la possibilité d'une présélection due à des différences de répulsivité à l'entrée des insectes dans les étables traitées.