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OBSERVATIONS ON THE DEVELOPMENT AND TREND OF INSECTICIDE RESISTANCE IN TWO IMPORTANT MALARIA VECTORS IN INDONESIA

by

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Introduction

The progress of the malaria eradication programme in Indonesia has been most satisfactory and the appearance of insecticide resistance in some parts of the country in A. aconitus and A. sundaicus has not impeded the programme to any extent. Observations on the distribution and variation of resistance to DDT or dieldrin are not complete but even at this stage of very great interest and value (Fig. 1).

I. DEVELOPMENT OF INSECTICIDE RESISTANCE IN A. ACONITUS DÖNITZ

A ricefield breeder, A. aconitus is the main malaria vector in the interior of Java. It was first found to be resistant to dieldrin in the Subah district of Central Java in October 1959 after three years of dieldrin spraying (six half-yearly cycles of 0.5 g/m^2). At this time the RR portion of the anopheline population was 64% of the whole. In February 1960, A. aconitus was found to be resistant to dieldrin in Jogjakarta, Central Java, in an area which had also been subject to three years of dieldrin spraying (one cycle per year of 0.5 g/m^2). Here the RR proportion was 36% and the LD_{50} for DDT at this time was 0.6%.

Thereafter dieldrin resistance was reported successively from:

Serang, West Java (July 1960)

Malang, East Java (February 1961) (This latter is interesting as the first demonstration in Indonesia of the development of resistance in a malaria vector purely by agricultural use of insecticides.) (Badawi, report to WHO, 1961.)

Lampung, South Sumatra (August 1961)
Banjuwangi, East Java (April 1962)
Klaten, Central Java (May 1962)
Bodjonegoro, East Java (May 1962)
Tjiandjur, West Java (May 1962)
Purworedjo, Central Java (November 1962)
Island of Madura, East Java (March 1963)
Magelang, Central Java (July 1963)

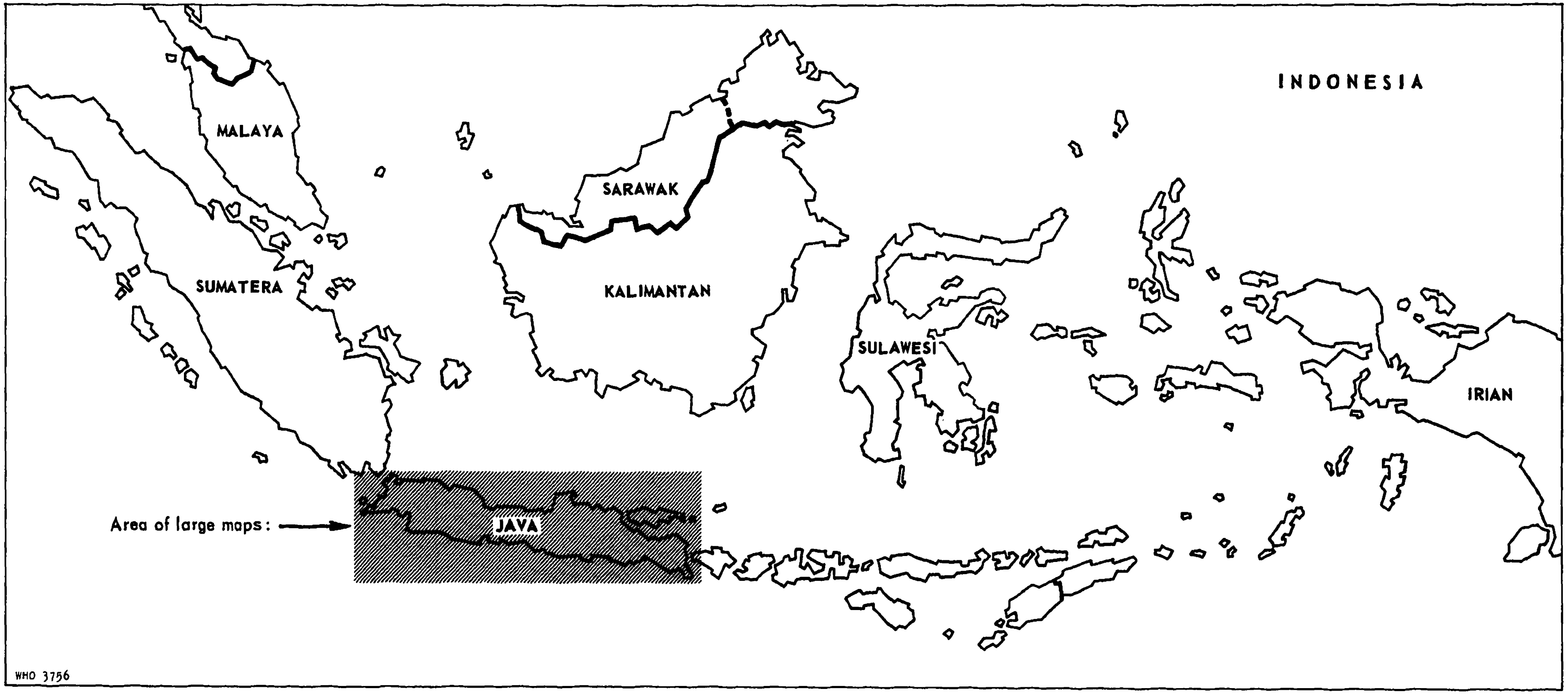
When it became apparent that dieldrin resistance was spreading in A. aconitus it was decided to limit the use of this insecticide by the malaria programme. Its use was accordingly restricted to areas where A. sundaicus was formerly found to be resistant to DDT and to areas where there are well-defined A. sundaicus breeding places. DDT is used in areas where there is no distinct boundary between A. aconitus and A. sundaicus.

In March 1962, A. aconitus suddenly appeared in high densities in Jogjakarta, Central Java. Susceptibility testing showed the vector to be resistant to both DDT and dieldrin with resistant homozygotes in the proportions of 73% and 46% respectively (assuming that discriminating doses for A. aconitus are similar to those demonstrated so far for other species). Spraying history in the area of Jogjakarta which gave the above figures was:

1957: dieldrin (0.5 g/m^2) one cycle.
1958, 1959: DDT (2 g/m^2) two cycles.
1960, 1961, 1962: DDT (1 g/m^2) four cycles.

In Bodjonegoro, East Java, in May 1962, an "intermediate" state of DDT resistance was demonstrated for this vector. This was also the position in May 1962 in the zones immediately surrounding Jogjakarta. Testing in March-July 1963, however, has demonstrated that the resistance in parts of these zones (Purworedjo, Magelang and Klaten) is now at a high level. In the central Jogjakarta area no increase in the level of DDT resistance was apparent between April 1962 and April 1963.

FIG. 1


INDONESIA
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In June and July 1962, an infant parasite survey was carried out in the Jogjakarta area. A total of 2641 slides were examined but no positives were found. During April, May and June 1962, active and passive case detection together with epidemiological surveys in the neighbourhood of confirmed cases produced 182 positive slides of which 72 were classified as indigenous. Total slides examined were 43 496.

During the first quarter of 1963 the number of slides examined in Jogjakarta amounted to 75 803 of which 42 were positive and 9 indigenous. In April 1963, 16 415 slides were examined of which 7 were positive and none classified as indigenous.

Thus, despite the occurrence of DDT resistance in A. aconitus in Jogjakarta, there is no indication so far of an increase in transmission of malaria. This situation appears to be due mainly to depletion of the parasite reservoir during the two years of eradication prior to the upsurge of resistance to a detectable level. Biting still takes place indoors and outdoors and can reach extremely high levels if the human bait is in the vicinity of cattle (especially water buffalo). No A. aconitus have so far been found resting on the DDT deposit.

Experimental hut observations were undertaken with a view to determining the effect of DDT on those A. aconitus entering sprayed structures in this area of DDT resistance, and also to compare this with the effect of malathion and a mixture of BHC with malathion. Considering freshly-fed females only, the DDT hut produced mortalities which fell rapidly from 15.7% in the second week after spray to 5.4% in the third week. Thereafter kills were extremely low, and during some weeks no mortality was produced at all.

The BHC/malathion mixture produced complete kills up to the sixth week, then mortalities fell off rapidly. After the seventh week the total catch dropped markedly in this hut, and no significance can be attached to the succeeding results.

The malathion at 2 g/m^2 produced complete mortalities for the first nine weeks, and the kill stayed above 83% for the remainder of the observation period. This was a total of 17 weeks after spray. By this time the catch size had declined in this hut also and the observations were temporarily halted.

Reduction in biting was most marked in the malathion-sprayed hut. On examining the proportions of unfed females produced by each hut the following results are obtained:

	Percentage of unfed females	Total catch
BHC/malathion	10	163
DDT	4.4	659
Malathion	13	943
Control	6.7	1515

The figure for the DDT hut is interesting in that it confirms the indication obtained in the trials with A. sundaicus pointing to a slight increase in biting in DDT-sprayed huts when compared with an unsprayed control. This is possibly due to the excitation of those unfed females which would normally only rest in an unsprayed hut and leave again in an unfed state (6.7% of total entry in the above observations with A. aconitus). An apparent repellency from the sprayed huts was suggested by a sudden increase in the control catch immediately after spray:

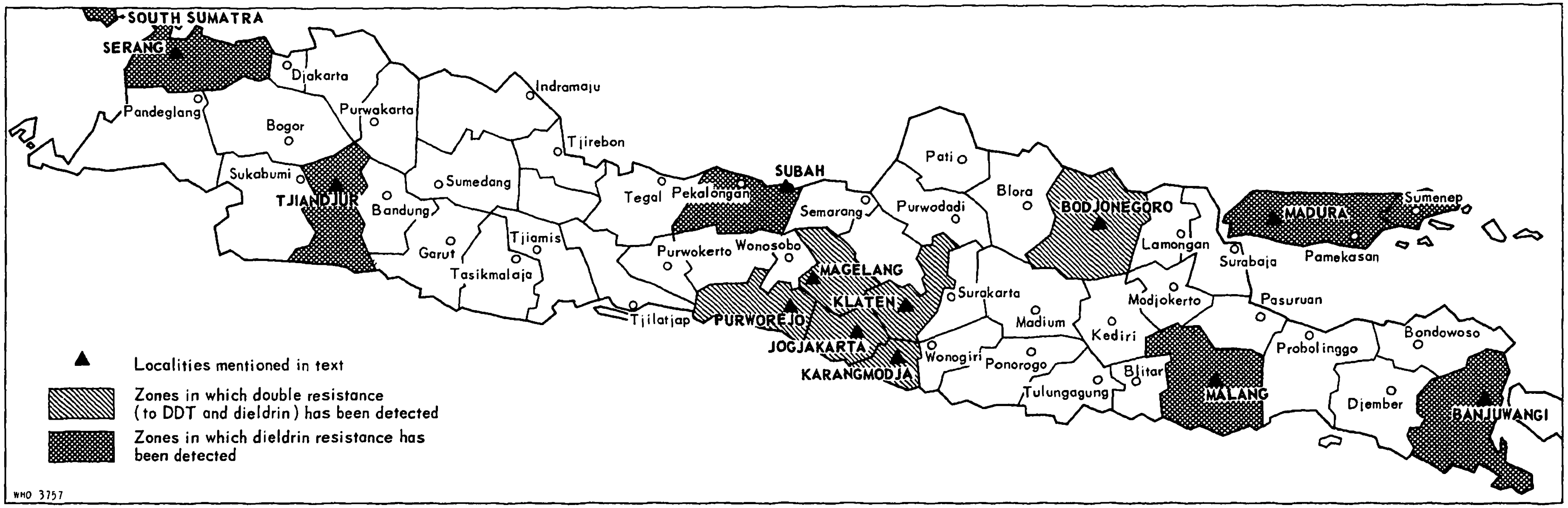
The two days before spray	The two days after spray
48	125

This is supported by the total catch figures given above, but further observations are necessary.

It was shown (by testing the survivors from the DDT hut) that selection was still operating (at least in the first two weeks after spray) on those A. aconitus which entered this sprayed structure. The question arises, however, as to why there has been no apparent increase in resistance level in this area over the past year. The answer may be that in Jogjakarta there is a very high deviation to cattle, especially to water buffalo, and consequently selection pressure has been of a fairly low order.

FIG. 2

DEVELOPMENT OF INSECTICIDE RESISTANCE IN *A. aconitus* Donitz IN JAVA



Since this deviation has always been operating it is possible that agricultural use of DDT has been mainly responsible for this high level of DDT resistance (Brown, 1962; Hamon & Garrett-Jones, 1963).

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II. DEVELOPMENT OF INSECTICIDE RESISTANCE IN *A. SUNDAICUS* RODENWALDT

Hamon & Garrett-Jones (1963) have written a comprehensive summary of the situation with regard to this species, and the present account is intended to be complementary to this.

Both DDT resistance and dieldrin resistance have been detected in *A. sundaicus* but never in the same population, and so far only in the Island of Java (recent unconfirmed reports indicate DDT resistance in South Sumatra and dieldrin resistance in Sarawak). In Java, DDT resistance has so far been confined to the North coast and dieldrin resistance to the South coast of the island (Fig. 3).

The first record is that of Crandell (1954) who demonstrated the presence of DDT resistance in Djakarta and Tjirebon. By 1955 resistance had also been detected in two other towns on the North coast of Java, Semarang and Surabaja, after two and four years respectively of use of residual DDT in the malaria control programme which started in 1952 (Chow & Soeparmo, 1956; Chow, 1958). Some aerial spraying with DDT had also taken place between 1945 and 1949.

Davidson (1957) showed that DDT resistance in *A. sundaicus* is monofactorial and almost completely recessive, an exposure for one hour to a 4% solution of DDT in Risella oil killing all SS and rS individuals and also 4% to 6% of rr.

After the development of resistance of *A. sundaicus* to DDT instructions were issued for the use of dieldrin (0.5 g/m^2) in coastal areas instead of DDT. Dieldrin gave excellent control of *A. sundaicus* in the above foci of DDT resistance until spraying was temporarily halted in 1958 during the change-over from a control programme to an eradication programme. In 1960, during this interruption of spray, the density of A. sundaicus in Semarang, Central Java, increased to such an extent that there was an outbreak of malaria. Man-biting ratios of 160 per night in houses and 250 per night outside were recorded (Soerono & Muir, 1963). Testing of this population showed no apparent resistance to DDT or to dieldrin, and the latter insecticide was used successfully (along with mass chemoprophylaxis) to control the outbreak. A. sundaicus has not been found in Semarang since this time, and dieldrin is still used in the eradication programme in this area.

The reasons for this apparent reversion to DDT susceptibility in Semarang are not clear, but the most likely explanation appears to be the immigration of the vector from unsprayed areas.

Dieldrin resistance in A. sundaicus has so far been found only on the South coast of Java. It was first detected in November 1959 on the coast of Jogjakarta, Central Java. This was before the beginning of eradication, but after two cycles of DDT and two of dieldrin under the control programme ending in 1958. When tested against dieldrin in 1959 susceptible homozygotes formed about 50% of this population. Further to the West, in South Kedu, the situation was similar at this time (52% susceptible homozygotes). Here control had consisted of two sprayings of dieldrin (0.25 g/m^2), one in 1957 and one in 1958. This South Kedu area remained unsprayed from 1958 until 1963, and successive tests in 1960, 1961 and 1962 indicated a progressive decrease of the dieldrin-resistant portion of the population until 1961, there being no progressive change from 1961 to 1962:

	1959	1960	1961	1962
Percentage of RR	8	6	0.5	0.5

Davidson (personal communication) has shown that dieldrin resistance in A. sundaicus is due to a semi-dominant allele, 4% dieldrin for one hour killing more than 90% of hybrids.

Still further to the West, near Tjilatjap, testing against dieldrin in 1961 indicated an RR of 3%. This area had received three yearly cycles of dieldrin (0.5 g/m^2) ending in 1958.

In March 1962 dieldrin resistance in _A. sundaicus_ was detected in Zone Garut, West Java (Adjahri, unpublished report), and in November 1962 in Zone Djember, East Java (Badawi, report to WHO, 1963).

The typical breeding-places of _A. sundaicus_ on the North coast of Java are man-made fishponds along the edge of the relatively calm Java Sea. On the South coast however they are salt marsh or "lagoon" areas associated with river mouths. These marshy areas are cut off from tidal influence during the dry season by the formation of a sand-bar at the river mouth. Because of these differences of breeding site, and since DDT resistance in _A. sundaicus_ has so far been confined to the North coast and dieldrin resistance to the South coast, it had been suggested that there might be some biological distinction between the two populations. Reciprocal crossing of the North and South coast strains in the Ross Institute, London, has shown them to be interfertile. The F_1 generation was fed and fertile eggs obtained (Davidson, personal communication).

Sundararaman (1958) considered that DDT spraying had failed in its objective, in those areas which he was investigating, due to a behaviouristic change of the vector. Whether or not such a change may take place, an indication of the irritant effect of DDT deposits on _A. sundaicus_ is given by the results of an irritability test (WHO Provisional Method) and also by experimental hut observations over a period of six months in an area which had received only two cycles of dieldrin (0.25 g/m^2) during 1957 and 1958, one cycle each year.

IRRITABILITY TEST: (WHO PROVISIONAL METHOD, STEPS A AND B)

Average time lapse in minutes before first take-off			Average number of take-offs in 15 minutes		
2% DDT	4% DDT	Control	2% DDT	4% DDT	Control
7.1 (1.4)	2.3 (0.7)	78 (11.8)	13.2 (2.2)	27.8 (4.3)	0.6 (0.3)

Figures in brackets are associated standard errors.

EXPERIMENTAL HUT OBSERVATIONS

Hut sprayed with DDT (2 g/m^2)

	Unfed	Fresh-fed	Half-gravid	Gravid	Total
Total catch	232 (32%)	458 (61.7%)	11 (1.5%)	36 (4.8%)	742
Window-trap	93 (37.4%)	119 (47.8%)	6 (2.4%)	31 (12.4%)	249

Percentage in window-trap was 33.6% of total catch.

Control hut, unsprayed

	Unfed	Fresh-fed	Half-gravid	Gravid	Total
Window-trap	674 (39.2%)	56 (3.2%)	66 (3.8%)	926 (53.8%)	1722

Percentage in window-trap was 99.8% of total catch.

The difference in total female catch indicates the possibility of repellency from the DDT hut. It will be noted, however, that as judged by the total production of unfed females on a proportion basis, there is no apparent reduction in biting among those A. sundaicus entering the sprayed hut.

In the insecticide trials carried out in the A. sundaicus area on the South coast of Central Java, dieldrin 0.5 g/m^2 and BHC 0.5 g/m^2 emerge as the most effective insecticides. Their use is, however, precluded in this area due to the presence of dieldrin resistance in the A. sundaicus population. BHC 0.5 g/m^2 + malathion 1 g/m^2 and malathion 2 g/m^2 come next with little to choose between them. Malathion 2 g/m^2 appears to be slightly better than the mixture over the first four months, and being operationally simpler to use would be the insecticide of choice for this area.

The selective effect, of the various experimental deposits, on the dieldrin resistant A. sundaicus population was estimated by exposing survivors (after a 12-hour observation period) to 0.4% dieldrin for one hour and observing for another 15 hours. The three deposits showing the highest degree of selection are BHC 0.5 g/m^2 (actual mortality in test 56%), dieldrin 0.5 g/m^2 (actual mortality in test 83.5%) and the mixture DDT 1 g/m^2 + dieldrin 0.25 g/m^2 (actual mortality in test 85.5%). BHC selects most strongly, presumably because of its fumigant action. The actual mortality among survivors from the malathion 2 g/m^2 hut was 94.8% (91.5% corrected) which would imply no selection of the allele conferring dieldrin resistance by this insecticide.

The trials included a comparison of huts sprayed with DDT at dosages of 2 g/m^2 and 1 g/m^2. Considering the total catches for six months' observations from the two DDT-sprayed huts and from the control hut, it is seen that the proportions of unfed females taken in the DDT huts are almost the same (DDT 2 g: 32%; DDT 1 g: 30.7%) but are slightly less than the proportion taken in the control hut (39.2%).

While there is, apparently, the same amount of biting in the DDT 2 g and DDT 1 g huts, there is a difference in escape to the window-trap and also in survival:

(a) From the DDT 2 g hut 26% of the freshly-fed females escape to the window-trap and 18.1% survive.

(b) From the DDT 1 g hut 15.4% of the freshly-fed females escape to the window-trap and 11.1% survive.

Thus the evidence suggests that:

(1) The irritant effect of DDT will not reduce biting of those A. sundaicus which enter a sprayed structure.

(2) There may, however, be a repellent effect which reduces entry.

(3) The higher dose of DDT has a greater irritant effect than the lower dose, and causes increased escape and increased survival.

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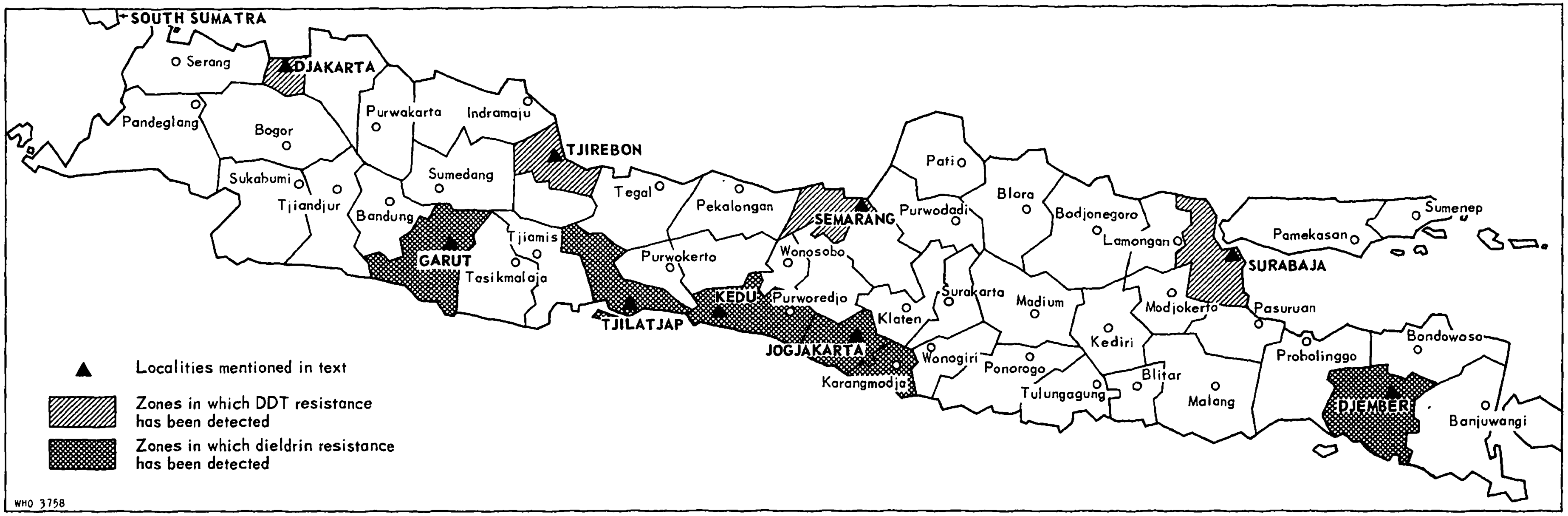
FIG. 3

DEVELOPMENT OF INSECTICIDE IN *A. sundaicus* Rodenwaldt IN JAVA



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