WARF ANTIRESISTANT COMPOUNDS AS SYNERGISTS AGAINST DDT-RESISTANT *AEDES AEGYPTI*

M. K. K. PILLAI, Z. H. ABEDI, AND A. W. A. BROWN
Department of Zoology, University of Western Ontario, London, Canada

The compound N-di-n-butyl-p-chlorobenzenesulfonamide, available under the name of WARF Antiresistant, has proved to be a highly effective DDT synergist against DDT-resistant house flies (Neeman, 1961). Added to DDT in ratios between 1:3 and 1:5, it has allowed the regular DDT-pyrethroid aerosols to obtain high kills of resistant flies (Fales and Bodenstein, 1961). Treatment of successive generations of the Fowler strain of house flies with WARF Antiresistant plus DDT in 1:1 ratio resulted in no increase in resistance to the mixture by the 9th generation (Wisconsin Alumni Research Foundation, 1961), but further selection gave an increase tolerance by the 16th generation which could be corrected by increasing the Antiresistant-DDT ratio to 5:1 (*Ibid.*, 1962).

The purpose of the following studies was to extend the tests to *Aedes aegypti,* using a 1:1 mixture. The first phase was to compare the susceptibilities to DDT and to Antiresistant-DDT mixture of 6 resistant strains and 5 susceptible strains. The second step was to submit 2 resistant and 1 susceptible strains to selection pressure from the Antiresistant-DDT mixture for 6 generations, to ascertain the speed at which resistance was developed (and could be subsequently lost) to the synergized mixture.

MATERIAL AND METHODS. The strains under investigation included 4 pairs of resistant and susceptible material of the same geographical origin, viz.: Trinidad R and a Trinidad S isolate (Brown and Abedi, 1962); Key West S and a developed Key West R (Abedi and Brown, 1961); New Orleans S and a developed New Orleans R (Abedi and Brown, 1961); Penang S and a developed Penang R (Abedi and Brown, 1960).

The 3 additional strains were as follows: Penang Malathion-R, whose DDT-resistance was developed by malathion selection (Brown and Abedi, 1960); Isla Verde R, resistant both to dieldrin and DDT (Khan and Brown, 1961); Guelph S, derived from a single Orlando female (Chattoraj and Brown, 1960).

All susceptibility tests were performed on larvae in the late 3rd or early 4th stadium, using the WHO standard method for mosquito larvae. Each strain was tested with DDT alone and with a 1:1 mixture of DDT and Anti-resistant; amyl-p-chlorobenzenesulfonamide (Amyl-antiresistant) was included in these tests as well as the regular butyl-p-chlorobenzenesulfonamide (Butyl-antiresistant).

The selection procedure also involved larvae of similar age, and was performed in the 16-oz. wide-mouth glass jars with 250 c.c. of water, similar to those used for the susceptibility tests, with a 24-hour exposure period. Selections were made with a 1:1 mixture of DDT and Butyl-antiresistant on each successive generation from the P to the F_6, with the exception of the F_1. The dosage was adjusted in each generation to give a selection pressure of approximately 80 percent mortality. Each generation was tested for its susceptibility level to Antiresistant-DDT mixture and to DDT alone. The strains submitted to selection were the Penang S, the Penang R and the Trinidad R. The F_6 generation was removed from selection pressure, and the susceptibility levels were determined for the strain relieved of selection from the F_7 to the F_{10} generation.

RESULTS. The susceptibility levels of the 11 strains to Antiresistant-DDT mixture and to DDT alone (Table 1) show that both the Antiresistant compounds are highly synergistic with DDT. Butyl-antiresistant reduces the LC_{50} of susceptible strains by 1 to 3 times, but of resistant strains 6 to 60 times. Amyl-antiresistant

TABLE 1.—LC_{50} levels in p.p.m. DDT of strains tested with DDT alone or with DDT plus Antiresistant in 1:1 mixture.

Strain	DDT alone	DDT plus Butyl-Antires.	DDT plus Amyl-Antires.
Trinidad R	1.50	0.18	0.47
Trinidad S	0.015	0.005	0.014
Key West R	1.00	0.09	0.11
Key West S	0.073	0.075	0.070
New Orleans R	10.2	0.55	1.00
New Orleans S	0.009	0.004	0.006
Penang DDT-R	15.0	0.26	0.43
Penang Malathion-R	0.60	0.10	0.12
Penang S	0.066	0.025	0.035
Isla Verde R	1.20	0.20	0.49
Guelph S	0.0076	0.0035	0.0047

reduces the LC_{50} of susceptible strains by 1 to 2 times, but of resistant strains by 2 to 30 times. It is clear that this synergistic activity, powerful against the resistant strains only, is stronger in the regular Butyl-antiresistant than in its Amyl-antiresistant analogue.

Selection of the Penang S Strain with Butyl-antiresistant plus DDT for 6 generations (Table 2) steadily increases the resistance to the mixture to reach 40 times the original, and increases the resistance to DDT alone by 20 times. Upon relaxation of selection pressure (Fig. 1) this resistance remained stable for 3 generations before significantly falling in the 4th, both for the mixture and for DDT alone.

Selection of the Penang R strain with the Antiresistant-DDT mixture (Fig. 2) showed an unexpected initial result: the resistance to the mixture diminished, and the LC_{50} to DDT alone decreased by 15 times. Subsequent to the F_2 generation, the resistance to DDT resumed its increase, and so did that to the mixture; so that by the F_6 it was approaching its original DDT-resistance level, and the increase in resistance to the mixture had become 8-fold. On relaxation of pressure the resistance remained stable for 3 generations before diminishing.

Selection of the Trinidad R strain (Fig. 3) gave similar results; in the initial 3 generations the LC_{50} levels to DDT de-

TABLE 2.—Susceptibility levels and selecting dosages in p.p.m. for successive generations selected by Antiresistant plus DDT in 1:1 mixture.

	Penang S			Penang R			Trinidad R		
Gen'n	Selecting dosage*	LC_{50} DDT	LC_{50}* anti-r	Selecting dosage*	LC_{50} DDT	LC_{50}* anti-r	Selecting dosage*	LC_{50} DDT	LC_{50}* anti-r
P	..	0.80	0.025	..	10.0	0.26	..	1.15	0.18
	0.045	..	..	0.75	..	..	0.5	..	..
F_1	..	..	..	..	..	..	..	..	..
	No selection			No selection			No selection		
F_2	..	0.11	0.075	..	0.62	0.20	..	0.41	0.14
	0.32	..	..	1.0	..	..	0.64	..	..
F_3	..	0.20	0.15	..	2.3	0.55	..	0.185	0.046
	1.0	..	..	2.56	..	..	0.5	..	..
F_4	..	0.41	0.165	..	5.6	0.34	..	1.1	0.47
	1.5	..	..	4.0	..	..	2.56	..	..
F_5	..	0.47	0.235	..	6.2	1.25	..	1.4	0.90
	2.5	..	..	6.0	..	..	5.12	..	..
F_6	..	1.5	1.05	..	6.2	2.0	..	5.4	2.05

* This figure applies simultaneously to DDT and to Antiresistant, each present in equal amounts.

creased by 8 times and to the mixture by 4 times, but subsequently they increased so that the final DDT-resistance was 4 times the original, and the resistance to the mixture had become more than 10-fold. On relaxation of pressure the resistance remained stable for 3 generations, and then fell by 3 to 5 times in the 4th generation.

DISCUSSION. It is clear that Butyl-antiresistant and its amyl analogue are strong DDT synergists for DDT-resistant larvae of *Aedes aegypti*. Tests which had been made on the same array of strains with DMC:DDT mixtures in the proportion 1:5 (Abedi, Duffy and Brown, 1963) showed that DMC in this ratio reduced the LC_{50} levels of resistant strains to

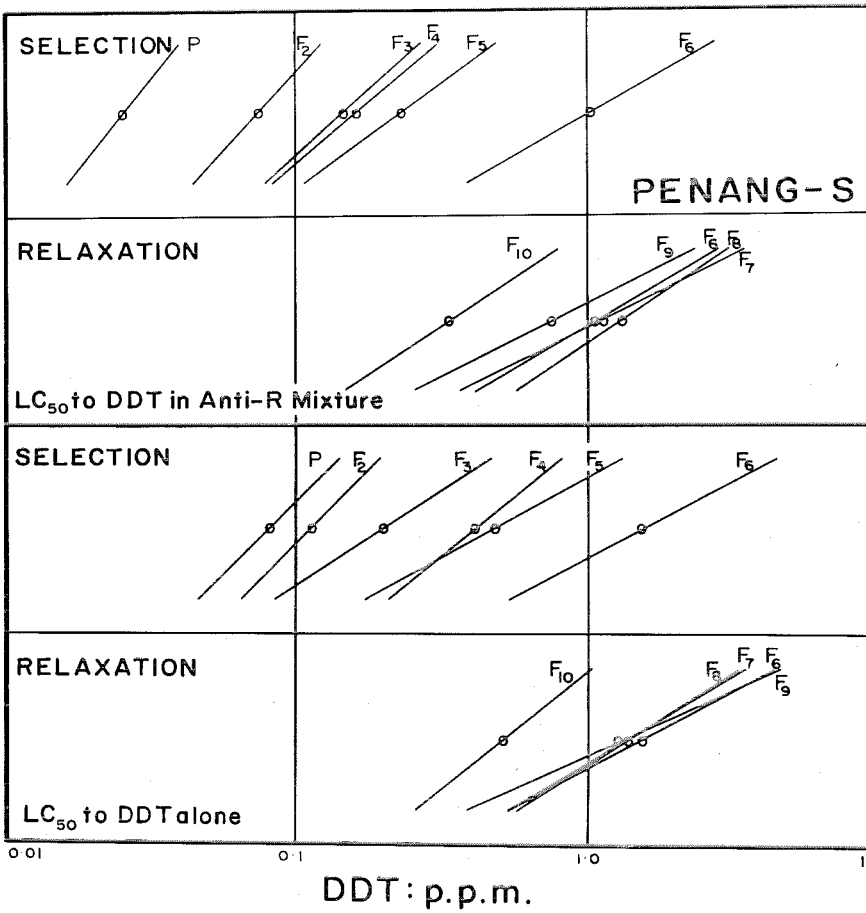

FIG. 1.—Selection of Penang-S strain with DDT plus Butyl-antiresistant: LC_{50} levels to DDT in the mixture and to DDT alone.

N.B. The dosage-mortality lines extend from 30 to 70 percent mortality.

DDT by 2 to 14 times; the present work shows that Butyl-antiresistant in a 1:1 ratio is about 4 times more potent than DMC in reducing the LC_{50} to DDT. The reason for the action of DMC on resistant house flies is that it inhibits the detoxifying DDT-dehydrochlorinase enzyme (Perry, Mattson and Buckner, 1953); Butyl-antiresistant also has been found to inhibit the house fly DDT-dehydrochlorinase when added to DDT in 1:10 ratio, but not in 1:100 (Wisconsin Alumni Research Foundation, 1962). Tests with DDT-dehydrochlorinase from *A. aegypti* performed by Mr. T. Kimura have shown that Butyl-antiresistant is inhibitory when added to DDT in a 1:1 ratio, but scarcely inhibitory in the 1:10 ratio at which DMC

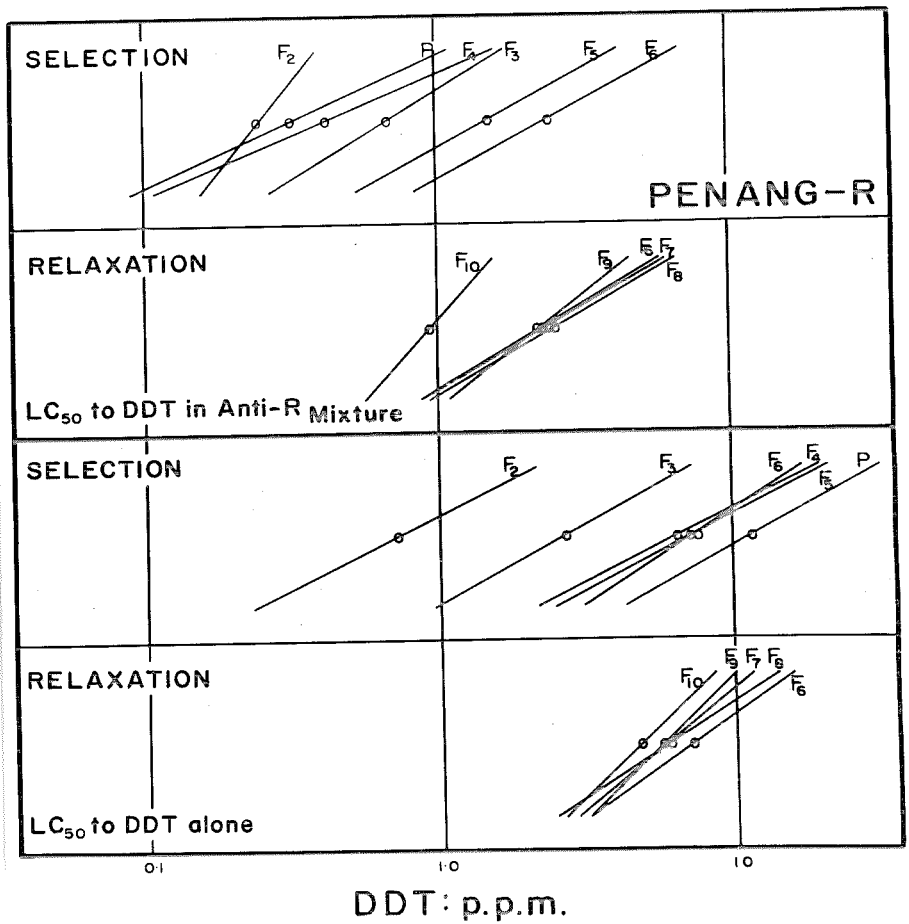

FIG. 2.—Selection of Penang-R strain with DDT plus Butyl-antiresistant: LC_{50} levels to DDT in the mixture and to DDT alone.

still markedly inhibits the mosquito enzyme.

It had been hoped that the admixture of Butyl-antiresistant to DDT would not result in a selection for resistance, since it had not done so in house flies, in contrast to other chlorinated hydrocarbon synergists such as K-3926 which had induced in 5 generations a resistance to the DDT in the mixture fully as great as the original resistance to DDT alone (March, Metcalf and Lewallen, 1952). But selection of *A. aegypti* with the Antiresistant-DDT mixture induced in 6 generations a resistance to the DDT in the mixture which in one resistant strain was almost as great, and in the other even greater, than the original resistance to DDT alone.

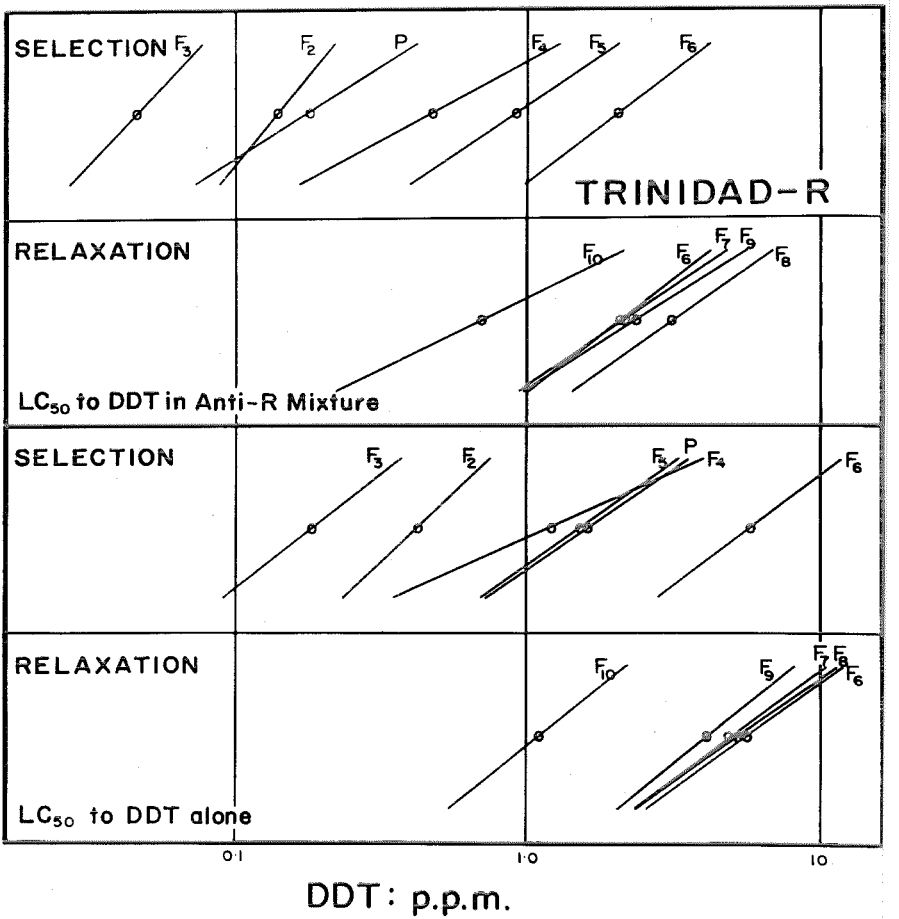

FIG. 3.—Selection of Trinidad-R strain with DDT plus Butyl-antiresistant: LC_{50} levels of DDT in the mixture and to DDT alone.

It is however of unusual interest that the Antiresistant-DDT mixture actually reduces the resistance in the first 2 or 3 generations of selection, suggesting that initially it selects for DDT-susceptibility. Thus there are possibilities that Butyl-antiresistant is a negatively-correlated compound with respect to DDT, and that the resistance developed to the Anti-resistant-DDT mixture is a different entity from the usual specific DDT-resistance.

SUMMARY. The effectiveness of WARF Antiresistant as a synergist for DDT was investigated on larvae of 6 resistant and 5 susceptible strains of *A. aegypti*. The Butyl-antiresistant decreased the LC_{50} levels of resistant strains to DDT by 6 to 60 times, and its Amyl-antiresistant analogue reduced them by 2 to 30 times. The synergistic effect was negligible with susceptible strains. Selection of a susceptible strain with a mixture of Butyl-antiresistant and DDT for 6 generations increased its resistance to DDT and the mixture by 20 and 40 times respectively. Similar selection of 2 resistant strains increased their resistance to the mixture by 8 to 10 times, so that the larvae by the 6th generation required about as much DDT in the mixture as had originally been required alone. However, the first 2 or 3 generations of selection had the unexpected effect of considerably reducing the resistance to DDT and to the mixture.

This work was performed on a grant-in-aid from the Wisconsin Alumni Research Foundation. The authors are indebted to Drs. M. Neeman and P.H. Derse for supplies of the Antiresistant compounds, and to Mr. T. Kimura for the tests of their effect on DDT-dehydrochlorinase.

References

ABEDI, Z. H., and BROWN, A. W. A. 1960. Development and reversion of DDT-resistance in *Aedes aegypti*. Can. Jour. Genet. Cytol. 2:252–261.

ABEDI, Z. H., and BROWN, A. W. A. 1961. DDT-resistance hazard in North American populations of *Aedes aegypti*. Mosquito News 21:1–4.

ABEDI, Z. H., DUFFY, J. R., and BROWN, A. W. A. 1962. Dehydrochlorination and DDT-resistance in *Aedes aegypti*. Jour. Econ. Ent. 56:(in press).

BROWN, A. W. A., and ABEDI, Z. H. 1960. Cross-resistance characteristics of a malathion-tolerant strain developed in *Aedes aegypti*. Mosquito News 20:118–124.

BROWN, A. W. A., and ABEDI, Z. H. 1962. Genetics of DDT-resistance in several strains of *Aedes aegypti*. Can. Jour. Genet. Cytol. 4:319–332.

CHATTORAJ, A. N., and BROWN, A. W. A. 1960. Internal DDE production by normal and DDT-resistant larvae of *Aedes aegypti*. Jour. Econ. Ent. 53:1049–1051.

FALES, J. H., and BODENSTEIN, O. F. 1961. Promising synergist for DDT. Soap Chem. Spec. 37(11):77 et seq.

KHAN, N. H., and BROWN, A. W. A. 1961. Genetical studies on dieldrin-resistance in *Aedes aegypti* and its cross-resistance to DDT. Bull. Wld. Hlth. Org. 24:519–526.

MARCH, R. B., METCALF, R. L., and LEWALLEN, L. L. 1952. Synergists for DDT against insecticide-resistant house flies. Jour. Econ. Ent. 45:851–860.

NEEMAN, M. 1961. Insecticidal compositions. United States Patent Office No. 2,974,083. March 7. 6 pp.

PERRY, A. S., MATTSON, A. M., and BUCKNER, A. J. 1953. The mechanism of synergistic action of DMC with DDT against resistant house flies. Biol. Bull. 104:426–438.

WISCONSIN ALUMNI RESEARCH FOUNDATION. 1961. WARF Antiresistant for DDT. Tech. Rep. W.A.R.F. (Madison 5, Wis.) No. N2–E2 (1260). 15 pp. mimeo.

WISCONSIN ALUMNI RESEARCH FOUNDATION. 1962. WARF Antiresistant for DDT, second report. Tech. Rep. W.A.R.F. No. N2–E3 (1161). 25 pp. mimeo.