Development of Resistance to Various Insecticides in *Culex pipiens fatigans* Wiedemann

T. TADANO & A. W. A. BROWN

Since larvicidal treatment to reduce the numbers of *Culex fatigans*, the tropical house mosquito, offers a promising means of preventing the spread of filariasis, an assessment was made of the liability of *C. fatigans* to develop resistance to certain important insecticides. Strains of this mosquito from Burma, Cameroon, Sierra Leone and the USA (California) were submitted to larval selection for periods of seven to 23 generations. It was found that resistance to DDT or deuto-DDT rapidly developed to high levels and that to dieldrin or CP-47412 to moderate levels; dieldrin did not select for DDT-resistance.

The organophosphorus compounds chosen developed only a modest tolerance—fivefold to sixfold with malathion or parathion, twofold to fourfold with fenthion or diazinon; the carbamate insecticide Hercules 5727 induced a threefold to fivefold tolerance. There was a general cross-tolerance from the selecting agent to all of the organophosphorus compounds tested and to the carbamate. A Ragoon strain that had already been made DDT-resistant subsequently developed a ninefold resistance to fenthion after 23 generations of selection with this organophosphorus compound, but this is still insufficient to annul the larvicidal effectiveness of fenthion.

Control operations against the tropical house mosquito *Culex pipiens fatigans*, vector of filariasis, have made use of the chlorinated hydrocarbon insecticides for larvicidal treatment and residual sprays against adults. This species is normally less susceptible to DDT than other mosquitoes, and can develop a decisive DDT-resistance. In India, this resistance developed after six years of adulticide treatment in Uttar Pradesh (Pal, Sharma & Krishnamurthy, 1952) and after only three years in Orissa (Krishnan, 1956). In Malaya, DDT-resistance in larvae necessitated the substitution of gamma-HCH or dieldrin for larval control (Reid, 1956). However, the introduction of dieldrin adulticide treatments in Andhra Pradesh induced a decisive dieldrin-resistance within three years (Naidu, 1962). Resistance to dieldrin and gamma-HCH has been developed by larvae of *C. fatigans* in many parts of the world.

Certain organophosphorus compounds, particularly malathion, parathion and diazinon, have been substituted. The introduction of malathion and parathion in 1951 to control *C. quinquefasciatus* (= *C. fatigans*) in California eventually led to control failures with malathion in Kern County, with a twofold to threefold increase in tolerance of para-thion (Barr, 1962). The introduction of malathion at Douala, Cameroon, in 1957 induced an 80-fold increase in the larval LC50 not only to malathion but also to diazinon (Mouchet et al., 1960). The use of malathion and diazinon in Freetown, Sierra Leone, increased the larval LC50 levels up to 0.5 ppm for malathion and 3.5 ppm for diazinon, which were respectively 10 and 50 times the normal levels.

The work of the WHO Filariasis Research Unit at Ragoon, Burma, has made it desirable to investigate the liability of *Culex fatigans* to develop resistance to the new organophosphorus and carbamate insecticides as well as to the chlorinated hydrocarbons. In the investigation here reported, strains from Ragoon and from Douala, Freetown and southern California were selected in the laboratory with DDT.

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dieldrin, four organophosphorus insecticides and one carbamate compound. Selection at the 90%–95% mortality level was applied to the larvae of each successive generation. In addition, for comparison, a substrain of the Rangoon strain was first selected for DDT-resistance and then submitted to selection with dieldrin, fenthion and the carbamate compound. Selection experiments were also performed with deuto-DDT and compound CP-47412 as remedial insecticides for DDT-resistance.

MATERIAL AND METHODS

The following strains of C. fatigans were submitted to selection:

Rangoon-N: derived from four females surviving from a field collection sent in May 1963.
Rangoon-LP: derived from about 100 females successfully shipped in December 1963.
Douala-N: derived from about 70 females field-collected and sent in November 1963.
Freetown-N: derived from about 500 females field-collected and brought to this laboratory by Dr J. Mouchet in December 1963.
Fresco-N: derived from about 150 females sent from a laboratory colony maintained at Fresno, Calif., and originally collected in Orange County, Calif.

Rangoon-DDT: a substrain of the Rangoon-N developed by two generations of selection with DDT and a further generation without selection, thus being the F3 generation.

All of the N (normal) strains were already somewhat DDT-resistant, especially the Freetown-N and Fresno-N. The Rangoon-DDT strain developed a 100-fold DDT-resistance as compared to the Rangoon-N strain. All strains except the Fresno-N also contained some dieldrin-resistant heterozygotes.

The insecticides employed were 95%–99.5% pure for the toxicant—namely, DDT (p,p' isomer) (OMS-16), dieldrin (99.5% HEOD) (OMS-14), gamma-HCH (OMS-17), malathion (OMS-1), diazinon (OMS-469), parathion (OMS-19), methyl parathion (OMS-213) and fenthion (OMS-2). The following additional insecticides, not characterized in the list of Kenaga (1963), were also employed:

- Brøoppers (O,O-dimethyl O-2,5-dichloro-4-bromophenyl phosphorothioate) (OMS-658)
- Abate (O,O',O'-tetramethyl O,O'-thiodi-p-phenylene phosphorothioate) (OMS-786)
- Hercules 5727 (N-methyl m-isopropylphényl carbamate (OMS-15)
- Deuto-DDT (2,2-bis (p-chlorophenyl)-1,1,1-trichloroethane-2-D) (OMS 867)
- CP-47412 (2,2-bis (p-chlorophenyl)1,1-dichlorocyclopropane) (OMS-1027)

Larval susceptibilities to these insecticides in ethanolic solution were determined by the standard method (WHO Expert Committee on Insecticides, 1963). The adult susceptibility levels were also tested by the WHO method for adult mosquitoes, unmodified for malathion, but with continuous exposure for DDT and dieldrin to obtain the LT₅₀ values (50% lethal time).

### Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rangoon-N</th>
<th>Rangoon-DDT</th>
<th>Rangoon-LP</th>
<th>Douala-N</th>
<th>Freetown-N</th>
<th>Fresno-N</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDT</td>
<td>0.14</td>
<td>13.5</td>
<td>0.64</td>
<td>0.73</td>
<td>1.60</td>
<td>3.40</td>
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<tr>
<td>Dieldrin</td>
<td>0.32</td>
<td>0.037</td>
<td>0.040</td>
<td>0.45</td>
<td>0.13</td>
<td>0.0085</td>
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<tr>
<td>Gamma-HCH</td>
<td>—</td>
<td>—</td>
<td>0.049</td>
<td>0.27</td>
<td>0.072</td>
<td>0.025</td>
</tr>
<tr>
<td>Malathion</td>
<td>0.056</td>
<td>0.058</td>
<td>0.038</td>
<td>0.064</td>
<td>0.068</td>
<td>0.043</td>
</tr>
<tr>
<td>Diazinon</td>
<td>0.058</td>
<td>0.055</td>
<td>0.049</td>
<td>0.076</td>
<td>0.080</td>
<td>0.068</td>
</tr>
<tr>
<td>Parathion</td>
<td>0.0037</td>
<td>0.0038</td>
<td>0.0026</td>
<td>0.0037</td>
<td>0.0037</td>
<td>0.0025</td>
</tr>
<tr>
<td>Methyl parathion</td>
<td>0.0047</td>
<td>0.0060</td>
<td>0.0034</td>
<td>0.0033</td>
<td>0.0033</td>
<td>0.0033</td>
</tr>
<tr>
<td>Fenthion</td>
<td>0.0025</td>
<td>0.0018</td>
<td>0.0023</td>
<td>0.0040</td>
<td>0.0025</td>
<td>0.0024</td>
</tr>
<tr>
<td>Bromophos</td>
<td>—</td>
<td>—</td>
<td>0.0041</td>
<td>0.0025</td>
<td>0.0024</td>
<td>0.0023</td>
</tr>
<tr>
<td>Abate</td>
<td>—</td>
<td>—</td>
<td>0.00037</td>
<td>0.00045</td>
<td>0.00032</td>
<td>0.00023</td>
</tr>
<tr>
<td>Hercules 5727</td>
<td>0.036</td>
<td>0.027</td>
<td>0.037</td>
<td>0.042</td>
<td>0.044</td>
<td>0.034</td>
</tr>
</tbody>
</table>
For selection of larvae, the insecticide was added in ethanolic solution to aliquots of 250 ml water in the glass jars used for the susceptibility tests; at least 1000 larvae were submitted to each selection, divided into lots of 100-200 in 12 jars. The concentration chosen for the selection was one that gave 90%-95% mortality; with dieldrin selection, however, the high concentrations necessary for the later generations were not increased beyond the 30% larval mortality level, to prevent the after-effect of dieldrin killing the pupae and the emerging adults. Most of the strain selections were continued for 10-20 successive generations. The LC50 levels were obtained from log-dosage-probit-mortality lines fitted by eye. The changes in LC50 levels were expressed as resistance ratios, calculated by dividing the final LC50 by the original starting LC50 (see Table 1). The starting LC50 of the Rangoon-DDT substrain was that of the F6 from its origin in the Rangoon strain.

RESULTS

When the Rangoon-N strain was selected with DDT (Fig. 1), the LC50 increased by 33 times in the F1, to attain a 280-fold increase by the F13 generation, when the LC50 reached the testable limit of 40 ppm. The successive dosage-mortality lines (Fig. 2) indicate that homozygosity for the main DDT-resistance gene had been achieved by the F10, in which all larvae survived a dose of 3 ppm; the subsequent increases in LC50 were largely due to the progressive flattening of the dosage-mortality line. The Fresno-N strain, which was already considerably DDT-resistant, responded to DDT selection by reaching an LC50 of 40 ppm in 11 generations. When these two selected strains in the F10 were tested for cross-resistance (Fig. 3), it was found

FIG. 2. DOSAGE-MORTALITY RELATIONSHIPS OF CERTAIN GENERATIONS OF THE RANGOON-N STRAIN SELECTED WITH DDT

FIG. 3. CROSS-RESISTANCE RATIOS TO VARIOUS INSECTICIDES OF TWO STRAINS SELECTED WITH DDT

--- Rangoon DDT-selected F10
--- Fresno DDT-selected F10

*Only the values for certain generations are shown; they are numbered accordingly.*
FIG. 4
DOSAGE-MORTALITY RELATIONSHIPS OF CERTAIN GENERATIONS OF THE RANGOON-N STRAIN SELECTED WITH DIELDRIN

Percentage Mortality

Rangoon
Dieldrin-selected

Dieldrin (ppm)

0.04 0.08 0.16 0.32 0.64 1.28 2.56 5.12 10.24 20.48 41

to be negligible to malathion, diazinon, parathion, methyl parathion and fenthion, and the cross-tolerance scarcely exceeded twofold to bromophos, Abate and the carbamate Hercules 5727, and to dieldrin and gamma-HCH.

Selection with dieldrin (Fig. 1) increased the LC$_{50}$ of the Rangoon, Douala and Freetown strains up to 5 ppm within six to eight generations, marking a 20-fold increase for the first two strains and a 40-fold increase for the initially more susceptible Freetown strain. Homozygosity for the main dieldrin-resistance gene was evidently attained in the first selection of the Rangoon strain, which removed the 33% of the larvae susceptible to the concentrations of 0.08 ppm to 0.32 ppm characteristic of heterozygotes (Fig. 4). The Fresno-N strain, which was initially very susceptible, failed to develop any real dieldrin-resistance in 10 generations of selection. When examined for cross-resistance (Fig. 5B), the first three strains were found to have increased their LC$_{50}$ to gamma-HCH by 3-10 times. There

FIG. 5
CROSS-RESISTANCE RATIOS TO VARIOUS INSECTICIDES OF STRAINS SELECTED WITH DIELDRIN a

A

B

C

\[
\text{Rangoon-\text{DDT} Dieldrin-selected } F_{10}
\]

\[
\text{Dieldrin-selected } F_{10}
\]

\[
\text{Freetown Dieldrin-selected } F_{6}
\]

\[
\text{Dieldrin-selected } F_{6}
\]

\[
\text{Dieldrin-selected } F_{10}
\]

\[
\text{Dieldrin-selected } F_{10}
\]

\[
\text{Rangoon Dieldrin-selected } F_{10}
\]

\[
\text{Dieldrin-selected } F_{10}
\]

a The Fresno strain did not develop dieldrin-resistance; the Rangoon-\text{DDT} strain had already been selected with DDT.
was no change in the LC\textsubscript{50} levels to diazinon, fen-thon, Abate and the carbamate, and an increase in susceptibility to malathion, parathion and methyl parathion. The LC\textsubscript{50} levels to DDT showed notable reductions, which were in direct proportion to the DDT-resistance level at the start of the dieldrin selection. The Fresno strain (Fig. 5A), in which there was no trace of dieldrin-resistance at either the start or the finish of the dieldrin selection, developed slight cross-tolerance to all the insecticides except malathion, but resembled the other strains in showing a reduction in its starting DDT-resistance.

Selection of the Rangoon-DDT strain with dieldrin increased the LC\textsubscript{50} to 15 ppm dieldrin in 11 generations, being a 50-fold increase. When the \( F_{10} \) was examined for cross-resistance (Fig. 5C), it was found that much of the DDT-resistance had been lost, and the tolerance levels to organophosphorus compounds and the carbamate had been slightly decreased.

Selection with malathion (Fig. 6) increased the larval LC\textsubscript{50} of the Douala and Freetown strains by 5-6 times, with no increase subsequent to the \( F_{6} \). The dosage-mortality lines (Fig. 7) remained parallel and steep, with slope values (increase in probit mortality per 10-fold increase in dosage) between 8 and 10. The cross-tolerances in the malathion-selected \( F_{10} \) generations were twofold to fourfold to diazinon, methyl parathion and the carbamate, and less than twofold to parathion, bromophos and Abate (Fig. 8A). Cross-tolerances to fenthion and gamma-HCH exceeded twofold only in the Freetown strain, in which the starting LC\textsubscript{50} had been lower than in the Douala strain. Both the malathion-selected strains showed a reduction in the LC\textsubscript{50} to DDT in direct proportion to the amount of pre-existing DDT-resistance, and the same thing happened in lesser degree to dieldrin.

Selection with diazinon (Fig. 6) increased the LC\textsubscript{50} by only 2-3 times, to reach a plateau at 0.16 ppm to 0.17 ppm diazinon by the \( F_{10} \) of the Fresno strain and by the \( F_{15} \) of the initially more susceptible Rangoon strain. The cross-tolerances in the \( F_{10} \) generations were slightly greater to malathion than to diazinon itself (Fig. 8B), and the LC\textsubscript{50} levels to fenthion, methyl parathion and the carbamate had risen even less than with malathion.
selection. However, the diazinon selection had increased the DDT-resistance level in both strains, but decreased the slight dieldrin-resistance in the Rangoon strain. Selection with parathion (Fig. 6) increased the LC$_{50}$ of the Rangoon-LP strain by 5 times with no increase subsequent to the F$_{16}$; it imparted only a slight cross-tolerance to all the other compounds (Fig. 8C).

Selection with fenthion (Fig. 6) increased the LC$_{50}$ by 4 times in 12-18 generations, the increase
being no greater in the highly susceptible Rangoon-LP than in the Douala strain. The tolerance increased steadily although at a gradually decelerating rate, and the dosage-mortality lines gradually steepened in slope from 5.5 to 9 (Fig. 7). Cross-tolerances were twofold to fourfold to malathion and diazinon, about twofold to bromophos and the carbamate, and slightly less to parathion, methyl parathion and Abate (Fig. 9A). However, the fenthion selection induced a 20-fold to 80-fold cross-resistance to DDT, in contrast to the slight cross-tolerance it developed to dieldrin and gamma-HCH.

Selection of the Rangoon-DDT strain with fenthion (Fig. 6) increased the LC₅₀ by 10 times in 22 generations. This high resistance ratio was partly due to the starting fenthion LC₅₀ being one-quarter lower in this DDT-selected strain, but was mainly due to the steady rise persisting as more generations were subjected to selection. The cross-resistance spectrum, as tested in the F₁₀ (Fig. 9B), resembled that developed in the Rangoon-N, namely, twofold to fourfold for malathion and diazinon and less than twofold for parathion and dieldrin; but the fenthion selection, far from inducing a cross-resistance to DDT, had instead reduced the pre-existing DDT-resistance which had been given to this Rangoon-DDT strain.

Selection with the carbamate Hercules 5727 (Fig. 10) increased the LC₅₀ of the Rangoon, Douala and Freetown strains by 4-5 times. There was no deceleration even after 16 generations of selection, and the dosage-mortality lines did not change in slope (Fig. 7). The cross-tolerances found in the F₁₀ were threefold to fourfold to malathion, diazinon and bromophos, twofold to fenthion and Abate, and less to parathion and methyl parathion (Fig. 11A). The LC₅₀ to DDT was increased slightly in the Rangoon and Douala strains and decreased in the initially more DDT-resistant Freetown strain. There was a twofold to fourfold increase in LC₅₀ to dieldrin, and a fourfold to 10-fold increase to gamma-HCH. Selection of the Rangoon-DDT strain (Fig. 10) produced a similar rate of increase in tolerance to this carbamate from a lower starting level. Cross-tolerances in the F₁₆ (Fig. 11B) were
FIG. 10. LC₅₀ LEVELS OF STRAINS SELECTED WITH THE CARBAMATE HERCULES 5727 OR WITH THE DDT-SUBSTITUTES DEUTERO-DDT AND CP-47412

Concentration of Insecticide (p.p.m.)

FIG. 11. CROSS-RESISTANCE RATIOS TO VARIOUS INSECTICIDES OF STRAINS SELECTED WITH THE CARBAMATE HERCULES 5727
less than 1.5-fold for diazinon, methyl parathion and fenthion, and less than unity for malathion and parathion. The initial DDT-resistance supplied to this strain was partially lost. But one effect of the carbamate selection on the Rangoon-DDT strain was to induce a 25-fold cross-resistance to dieldrin.

Selection with deuterodDTT (Fig. 10) increased the LC$_{50}$ of the Rangoon-LP strain by 240 times in eight generations, and this level remained stable for two subsequent unselected generations. DeuterodDTT selection of the Rangoon-DDT strain increased the LC$_{50}$ by 25 times in four generations to a level 100 times higher than that of the Rangoon-N strain. The cross-resistance spectrum of the selected Rangoon-LP strain showed only slight increases in LC$_{50}$ to all the organophosphorus compounds and gamma-HCH, but there was a 240-fold increase in cross-resistance to DDT, equal to that to the selecting agent deuterodDDT (Fig. 12). Conversely, the Rangoon DDT-selected strain developed less cross-resistance to deuterodDDT than to DDT, although in the Fresno strain the cross-resistances were about equal (Fig. 13).

Selection with CP-47412 (Fig. 10) raised the LC$_{50}$ of the Rangoon-LP strain by 60 times in seven generations. The dosage-mortality lines were steeper than those for DDT, but at the F$_6$ and F$_7$ a plateau of non-kill developed at the 75% mortality level. This compound was about 500 times as larvicidal as DDT whatever the level of DDT-resistance for all strains except the Freetown-N (Table 2).

The effect of larval selection on adult susceptibility levels was determined by submitting the F$_{10}$ of the malathion-selected Douala and Freetown strains and the original strains to the standard method for adult mosquitoes (WHO Expert Committee on Insecticides, 1963). The increase in adult LC$_{50}$ was found to be 1.7 to 2.5 times for the fivefold to sixfold increases in larval LC$_{50}$, as the following figures show:

<table>
<thead>
<tr>
<th>Larval strain</th>
<th>DDT</th>
<th>CP-47412</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rangoon-LP</td>
<td>0.64</td>
<td>0.0021</td>
</tr>
<tr>
<td>Freetown-N</td>
<td>1.60</td>
<td>0.089</td>
</tr>
<tr>
<td>Fresno-N</td>
<td>3.40</td>
<td>0.0075</td>
</tr>
<tr>
<td>Fresno, DDT-sel. F$_6$</td>
<td>20.0</td>
<td>0.055</td>
</tr>
<tr>
<td>Rangoon, DDT-sel. F$_{10}$</td>
<td>32.0</td>
<td>0.036</td>
</tr>
</tbody>
</table>

**TABLE 2**

**LC$_{50}$ LEVELS (ppm) OF C. FATIGANS LARVAE TO CP-47412 AS COMPARED WITH DDT**

<table>
<thead>
<tr>
<th>Larvae (ppm)</th>
<th>( %)</th>
<th>( %)</th>
<th>( %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Douala strain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LC$_{50}$, Normal</td>
<td>0.064</td>
<td>1.1</td>
<td>0.6</td>
</tr>
<tr>
<td>LC$<em>{50}$, Sel. F$</em>{10}$</td>
<td>0.41</td>
<td>1.85</td>
<td>1.5</td>
</tr>
<tr>
<td>Resistance ratio</td>
<td>6.4</td>
<td>1.7</td>
<td>2.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Larvae (ppm)</th>
<th>( %)</th>
<th>( %)</th>
<th>( %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freetown strain</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>LC$_{50}$, Normal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistance ratio</td>
<td>6.4</td>
<td>1.7</td>
<td>2.5</td>
</tr>
</tbody>
</table>
TABLE 3
RESISTANCE AND CROSS-RESISTANCE RATIOS FOUND AFTER 10 GENERATIONS OF SELECTION

<table>
<thead>
<tr>
<th>Selecting agent</th>
<th>Strain</th>
<th>DDT</th>
<th>Dieldrin</th>
<th>γ-HCH</th>
<th>Malathion</th>
<th>Diazinon</th>
<th>Parathion</th>
<th>Fenthion</th>
<th>Hercules 5727</th>
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</thead>
<tbody>
<tr>
<td>DDT</td>
<td>Rangoon</td>
<td>190</td>
<td>1.8</td>
<td>—</td>
<td>0.8</td>
<td>0.9</td>
<td>1.0</td>
<td>1.0</td>
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<tr>
<td></td>
<td>Fresno</td>
<td>8</td>
<td>1.8</td>
<td>2.5</td>
<td>1.4</td>
<td>1.2</td>
<td>1.1</td>
<td>1.2</td>
<td>1.6</td>
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<tr>
<td>Dieldrin</td>
<td>Rangoon</td>
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<td>—</td>
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<td>0.9</td>
<td>0.7</td>
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<td>1.1</td>
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<tr>
<td></td>
<td>Fresno</td>
<td>0.2</td>
<td>3</td>
<td>1.7</td>
<td>0.9</td>
<td>2.2</td>
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<td>1.4</td>
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<td>Malathion</td>
<td>Douala</td>
<td>0.5</td>
<td>0.5</td>
<td>1.4</td>
<td>6.4</td>
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<td>1.2</td>
<td>1.2</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>Freetown</td>
<td>0.4</td>
<td>0.8</td>
<td>3.3</td>
<td>5.1</td>
<td>3.9</td>
<td>1.7</td>
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<td>3.6</td>
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<tr>
<td>Diazinon</td>
<td>Rangoon (F₁₅)</td>
<td>2.3</td>
<td>0.2</td>
<td>—</td>
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<td>2.9</td>
<td>0.8</td>
<td>1.7</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>Fresno</td>
<td>1.4</td>
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<td>5.5</td>
<td>2.4</td>
<td>1.6</td>
<td>1.2</td>
<td>2.7</td>
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<td>Rangoon-LP</td>
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<td>1.3</td>
<td>—</td>
<td>1.1</td>
<td>1.5</td>
<td>4.6</td>
<td>1.5</td>
<td>1.1</td>
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<tr>
<td>Fenthion</td>
<td>Rangoon-LP</td>
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<td>1.6</td>
<td>1.8</td>
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<td>2.0</td>
<td>1.8</td>
<td>1.8</td>
<td>2.0</td>
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<td></td>
<td>Douala</td>
<td>14</td>
<td>1.8</td>
<td>1.6</td>
<td>2.3</td>
<td>3.3</td>
<td>1.5</td>
<td>3.3</td>
<td>1.4</td>
</tr>
<tr>
<td>Hercules 5727</td>
<td>Rangoon</td>
<td>1.6</td>
<td>2.5</td>
<td>—</td>
<td>2.9</td>
<td>3.6</td>
<td>1.3</td>
<td>1.3</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>Douala</td>
<td>2.0</td>
<td>2.1</td>
<td>3.6</td>
<td>2.5</td>
<td>3.0</td>
<td>1.3</td>
<td>2.1</td>
<td>3.8</td>
</tr>
</tbody>
</table>

Adult susceptibility levels to DDT were assessed with 4% DDT papers by the method of continuous exposure, since one-hour exposure had been insufficient to give any mortality in resistant strains. By this method the F₁₅ adults of the DDT-selected Rangoon strain showed an LT₅₀ of approximately 250 hours for both sexes, whereas the normal Rangoon-LP strain showed LT₅₀ values of 9 hours for males and 16 hours for females with an average of 12.5 hours. This 20-fold difference in the adults is to be compared with the 50-fold difference found in the larvae.

Likewise the standard one-hour exposure to 4% dieldrin papers caused no mortality in the dieldrin-selected Rangoon, Douala and Freetown strains. Continuous exposure gave a greater interstrain differential when 1.6% dieldrin papers were used, and by this method the F₁₅ adults of the dieldrin-selected Rangoon strain showed an LT₅₀ of approximately 20 hours for both sexes, whereas the normal Rangoon-LP strain showed an LT₅₀ of approximately two hours for both sexes. This 10-fold difference in the adults is to be compared with the 120-fold difference found between the F₁₅ dieldrin-selected larvae and the susceptible Rangoon-LP larvae.

DISCUSSION

The Rangoon-N and Rangoon-LP strains were already partially DDT-resistant and dieldrin-resistant, their LC₅₀ levels (0.14 ppm to 0.64 ppm DDT; 0.04 ppm to 0.32 ppm dieldrin) being quite representative of the LC₅₀ levels found in field samples in various parts of the city (ranging from 0.0025 ppm to 4.0 ppm DDT, and from 0.0006 ppm to 0.72 ppm dieldrin) which had been developed by DDT applications in 1945 and from 1950 to 1962 and the use of gamma-HCH in 1960 and 1961. Consequently the Rangoon-N strain responded very rapidly to larval selection with DDT. Yet the strong DDT-resistance produced did not carry any significant cross-resistance (Table 3) to the organophosphorus compounds and the carbamate insecticide or to dieldrin and gamma-HCH. Similarly, Hamon (1953) had found that the application of DDT for four years on the island of La Réunion produced strongly DDT-resistant larvae of C. fatigans with only slight cross-tolerance to HCH. It is true that 80 generations of laboratory selection with DDT, applied to a strain from East Nilgiris, South India, induced considerable cross-resistance to dieldrin and gamma-HCH (Mohan, 1960); but, although he found this strain to be pure for DDT-resistance, Davidson (1964) concluded it to be "not homozygous for dieldrin-resistance". On the other hand, selection of a field-collected strain from Kanpur induced strong resistance to DDT with no cross-resistance to gamma-HCH (Koshi, Dixit & Perti, 1963).

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The Rangoon-N strain also responded rapidly to dieldrin selection, as did the African strains, but the increases in LC50 were not so great as with DDT. These strains developed no more cross-resistance (Table 3) to the organophosphorus and carbamate compounds than the Fresno strain, which evidently contained no dieldrin-resistance genes. All four dieldrin-selected strains lost much of their pre-existing DDT-resistance. Field populations in East Pahang, Malaya, submitted to dieldrin residual adulticides for three years had developed only a twofold larval DDT-resistance as compared to the 60-fold larval dieldrin-resistance (Wharton, 1958). Larvae of C. fatigans on Penang Island, Malaya, combated with gamma-HCH for two years, showed only a 10\% increase in their LC50 to DDT in contrast to their 10-fold dieldrin-resistance (Reid, 1955). Similarly the HCH-resistant population produced after 20 months of gamma-HCH larvicides at Kumbakonam, Madras, showed a DDT-tolerance which, though appreciable, was entirely lost in two subsequent generations without insecticide (Rajagopalan et al., 1954).

The Douala and Freetown strains came from localities where field resistance to malathion and diazinon had been reported, but yet they showed LC50 levels of only 0.07 ppm malathion and 0.08 ppm diazinon, similar to those found on the spot when they were collected in late 1963, but quite different from the LC50 levels of 1.8 ppm malathion and 1.6 ppm diazinon reported from Douala in 1960, and of 0.5 ppm malathion and 3.5 ppm diazinon encountered at Freetown in 1962. Laboratory selection of these and the other strains with the organophosphorus compounds for 10-20 generations induced only tolerance rather than resistance. Malathion and parathion induced tolerance more rapidly than diazinon and fenthion, the resistance ratios in the F10 being 5-6 for the first two, and 2-4 for the last two.

Selection with any one of these organophosphorus compounds induced a degree of cross-tolerance to any of the others (Table 3). Parathion seemed to suffer the least from cross-tolerance, and malathion the most; Abate suffered less than bromophos. Malathion pressure on the African strains increased the tolerance to diazinon about half as much as to malathion itself; this resembles the situation that developed at Douala in 1960, but not that at Freetown, where the use of malathion induced a much stronger resistance to diazinon. On the other hand, diazinon pressure on the Rangoon and Fresno strains increased the tolerance to malathion more than to diazinon itself. Evidently C. fatigans does not show the dichotomy in organophosphorus-resistance between methyl-substituted and ethyl-substituted compounds that Busvine (1959) had found in the housefly. It is true that selection with the ethyl-substituted parathion did not induce as much tolerance to methyl parathion as to parathion itself, but the same held good for selection with the methyl-substituted fenthion. On the other hand, a greater cross-tolerance to methyl parathion than to parathion is induced not only by the methyl-substituted malathion, but also by the ethyl-substituted diazinon. Not only is there no evidence of the methyl-ethyl dichotomy of organophosphorus-resistance in C. fatigans, but also there is no difference in cross-resistance spectra between the phosphorodithioate malathion with its carboxyester groups and the phosphorothioates diazinon and fenthion that lack such groups.

The cross-resistance spectrum of organophosphorus-resistance in C. fatigans may be compared with that of other culicine mosquitoes in the case of parathion selection. This induced tolerances to malathion, methyl parathion and fenthion which were less than half that to parathion. This contrasts with parathion selection in Aedes aegypti, which induced as much tolerance to malathion as to parathion itself (Matsumura & Brown, 1963). On the other hand, it resembles the effect of field applications of parathion on Aedes nigromaculis in California, where the resulting 100-fold resistance to parathion involved 10-fold cross-resistances to malathion and methyl parathion, and a fivefold cross-tolerance to fenthion (Brown et al., 1963).

Malathion selection pressure significantly decreased what DDT-resistance pre-existed in the strains of C. fatigans employed, while fenthion pressure increased it by 15-20 times. The pre-existing dieldrin-resistance in these strains was also decreased by malathion and increased by fenthion pressure. This contrasts with the effect of malathion selection in Aedes aegypti, which increased the dieldrin LC50 by 20\% and the DDT LC50 by 7-32 times (Brown & Abedi, 1960; Matsumura & Brown, 1963).

Selection with the carbamate Hercules 5727 raised the LC50 of the Rangoon, Douala and Freetown strains by 3-5 times in 10-16 generations, a much greater change than the mere twofold resist-

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ance induced by this compound on a California strain in 30 generations (Georghiou & Metcalf, 1963). However, it is only half as great as the 11-fold resistance developed in this strain by selection for 17 generations with its close relative aprocarb (Georghiou, 1965). The cross-resistance spectrum induced in C. fatigans by the carbamate Hercules 5727 (Table 3) was similar to that induced by the organophosphorus compounds. Approximately twofold cross-tolerances were produced to DDT and dieldrin in our strains, as compared with the complete lack of cross-tolerance to these chlorinated hydrocarbons found in the California strain (Georghiou & Metcalf, 1963). But the situation in C. fatigans is different from that in the housefly, which developed a high resistance to DDT and dieldrin after 40-55 generations of selection with Hercules 5727 (Georghiou, 1962).

The pre-selection of the Rangoon strain with DDT made it quicker to develop resistance to dieldrin or to fenthion, but not to the carbamate Hercules 5727, subsequently applied. The dieldrin-resistance was developed three times faster, and the fenthion-tolerance about 25% faster. This resulted in the Rangoon-DDT strain developing a ninefold fenthion-tolerance, but, since even then the LC₅₀ was below 0.02 ppm, it would not prevent fenthion being still practically effective as a larvicide. Moreover, the pre-developed DDT-resistance was largely lost during the subsequent selection with dieldrin or the carbamate; even the fenthion selection, which had developed some DDT-resistance in the normal strains, slightly decreased the DDT LC₅₀ level of the Rangoon-DDT strain. The cross-resistance pattern induced by these three insecticides in the Rangoon-DDT strain was no different from what they induced in the normal Rangoon strains, with one notable exception: subsequent carbamate selection of the Rangoon-DDT induced a 22-fold dieldrin-resistance as compared with the fourfold increase in LC₅₀ it induced in the normal Rangoon strain. Fenthion selection, which had caused a twofold increase in dieldrin LC₅₀ in the normal Rangoon strain, did not increase the dieldrin-tolerance of the Rangoon-DDT strain. This effect of carbamate selection contrasts with the inability of Hercules 5727 to increase the dieldrin-tolerance of the Riverside strain of C. fatigans, and with the ability of this compound to abolish the dieldrin-resistance and DDT-tolerance pre-existing in the Panama strain of Anopheles albimanus (Georghiou & Metcalf, 1963).

Selection of C. fatigans with deuto-DDT induces resistance to this remedial insecticide as fast as the cross-resistance to DDT (240-fold to both insecticides in eight generations), in contrast to Aedes aegypti, in which resistance to deuto-DDT develops more slowly than the cross-resistance to DDT (22-fold as against 670-fold in 10 generations). On the other hand, DDT selection induces slightly less cross-resistance to deuto-DDT in C. fatigans than in Aedes aegypti (2.5-fold as against fourfold for a 10-fold increase in DDT-resistance). Moreover, the differential (ratio) between the effectiveness of deuto-DDT and that of DDT itself is considerably greater in C. fatigans than in Aedes aegypti, as demonstrated by the following LC₅₀ figures from the Rangoon strain of C. fatigans as compared with the Trinidad strain of Aedes aegypti (Pillai et al., 1963):

<table>
<thead>
<tr>
<th></th>
<th>Aedes aegypti</th>
<th>C. fatigans</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d-DDT DDT Ratio</td>
<td>d-DDT DDT Ratio</td>
</tr>
<tr>
<td>Susceptible</td>
<td>0.008 0.013</td>
<td>1.6× 0.5</td>
</tr>
<tr>
<td>DDT-resistant</td>
<td>0.4 15 38×</td>
<td>0.16 22</td>
</tr>
</tbody>
</table>

However, in both species the toxicity of deuto-DDT to the DDT-resistant strains has decreased to LC₅₀ levels (between 0.1 ppm and 0.5 ppm) which are on the borderline of practical use. On the other hand, the compound CP-47412 is so much more larvicidal than DDT to C. fatigans that, even although the differential (ratio) is scarcely greater in DDT-resistant than in susceptible strains—namely, CP-47412 DDT Ratio

|                      | Susceptible | 0.002 0.06 | 300× |
|                      | DDT-resistant | 0.05 20 | 400× |

yet the LC₅₀ levels to this compound are still sufficiently low (0.05 ppm) for the compound to be practically effective. Moreover CP-47412 did not select for resistance as fast as DDT or deuto-DDT.


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RéSUMÉ

Des souches de *Culex fatigans* provenant de Rangoon (Birmanie), Douala (Cameroun), Freetown (Sierra Leone) et Fresno (Californie) ont été soumises à l'action de divers insecticides en vue d'étudier les modalités d'apparition et de développement de la résistance. La sélection des individus résistants, poursuivie pendant 7 à 23 générations, a été opérée par application à chacune d'entre elles de la concentration d'insecticide provoquant une mortalité larvaire de 90 %-95 %.

La résistance au DDT s'est développée rapidement et, à la 13e génération, la CL50 atteignait 280 fois sa valeur initiale. Des résultats analogues ont été obtenus avec le deutéro-DDT, cependant que la résistance au CP-47412 ne s'accroissait que dans une proportion moindre. La résistance à la dieldrine de toutes les souches, à l'exception de la souche californienne, a augmenté de 20-40 fois. On n'a observé aucun degré notable de résistance croisée aux organo-phosphorés après sélection par le DDT, le deutéro-DDT et la dieldrine. En ce qui concerne les organo-phosphorés, l'accroissement de la résistance a été de 5-6 fois (malathion et parathion) et de 2-4 fois (fenthion et diazinon) la valeur initiale. On a noté l'apparition d'une certaine résistance croisée à tous les organo-phosphorés et à un carbamate, l'Hercules 5727, après sélection par l'un quelconque de ces composés. Elle se manifestait particulièremment à l'égard du malathion, à un moindre degré vis-à-vis du parathion, et était également moins nette pour l'abate que pour le bromophos. La sélection par l'Hercules 5727 a déterminé un accroissement de 3-5 fois la valeur de la CL50 après 10-16 générations et a donné naissance à une résistance croisée aux organo-phosphorés.

Après avoir été rendue résistante au DDT, la souche birmane de *Culex fatigans* s'est montrée plus apte à acquérir une résistance au fenthion et à la dieldrine, mais non à l'Hercules 5727. Cette même souche, après sélection par le fenthion durant 23 générations, a témoigné d'une résistance 9 fois plus élevée qu'à l'origine envers cet organo-phosphoré, mais insuffisante cependant pour annuler complètement l'action larvicide du composé. Après exposition au carbamate, une diminution de la sensibilité à la dieldrine a été observée, notamment chez la souche DDT-résistante. Quant à la sélection par le fenthion, elle a entraîné une augmentation de la tolérance au DDT chez la souche normale, mais a réduit en revanche cette même tolérance chez la souche résistante au DDT.

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