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Resistance to Insecticides in the SKA Strain of Houseflies

R. M. SAWICKI

Historical

The study of the resistance of houseflies to organophosphorus (OP) insecticides was started at Rothamsted principally to increase the understanding of their mode of action. In 1955, two organophosphorus-resistant strains were obtained from Denmark and Italy, the two countries in Europe where resistance to both organochlorine and organophosphorus insecticides had first developed, and where control was difficult.

First results proved disappointing because the insects rapidly lost resistance. Additional resistant strains were therefore obtained from Denmark in December 1957, and from Italy in January 1958 and were crossed in autumn of the same year. The progeny of this cross gave rise to strain SKA—the subject of continuous research at Rothamsted for more than ten years.

Origin of strain SKA. Strain SKA was derived from the Latina strain and strain 203a.

Strain 203a. Strain 203a was collected in 1957 in a calf barn at Hilleröd, Sjaelland, Denmark, where flies had developed resistance in turn to DDT, chlordane and diazinon (Keiding, 1958, personal communication) and was kindly sent to us by Dr. J. Keiding of Lyngby. The females of F₁, the first generation after receipt were about 30 times more resistant to diazinon than our susceptible strain obtained from the Cooper Technical Bureau, Berkhamsted, Herts, when tested by the topical application of measured drops of insecticide in acetone. The strain was selected with diazinon from F₂ at every generation and maintained a fairly steady level of resistance (resistance factor (R.F.) = × 20). An unselected sub-strain of 203a lost its resistance within three to four generations.

Latina strain. The Latina strain kindly sent by Professor Saccà of Istituto Superiore di Sanità, Rome, in January 1958 originally came from the province of Latina where houseflies had become resistant to diazinon in the early 1950s. It was less resistant on receipt than strain 203a (R.F. *c.* × 15) and its resistance decreased progressively to about × 10 in spite of selection with diazinon at every generation. Unselected flies of Latina strain lost resistance much more slowly than flies of strain 203a.

Strain SKA. In November 1958 strains 203a F₁₉ and Latina F₁₇ were crossed in reciprocal crosses. Resistance between the two F₁ progenies differed little (R.F. 12–14) and was intermediate between that of the parents. The LD50s for diazinon of the parents and F₁ progenies were:

Latina F ₁₇	0.27 μg/♀
203a F ₁₉	1.35 μg/♀
F ₁ (203a × Latina)	0.65 μg/♀
F ₁ (Latina × 203a)	0.70 μg/♀

F₃ flies of the progenies of the two reciprocal crosses, which by then had lost some resistance (R.F. 6–9), were selected and the survivors mixed. The next generation (F₄) was named the SKA strain.

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Selection of only pregnant females at each generation increased resistance to $\times 30-40$, with a maximum R.F. of $\times 60$. Resistance increased considerably (to over $\times 200$) when virgin flies of both sexes were selected by topical application of measured drops of diazinon in acetone. The higher level of resistance was maintained when the dipping technique was substituted for topical application (Sawicki & Farnham, 1964), and after several years of selection little resistance was lost even when the flies were unselected for five or more generations. However in spite of very strong selection the strain was probably never completely homogeneous for resistance to diazinon.

SKA strain

Cross tolerance pattern. Table 1 shows the response of the SKA strain to 19 organophosphorus insecticides. It ranged from near susceptibility to almost complete tolerance

TABLE 1
Response of susceptible and SKA flies to some topically applied organophosphorus insecticides

Insecticide	LD ₅₀ $\mu\text{g}/\text{fly}$		Resistance factor
	Susceptible	SKA	
diazinon	0.050	10.47	209
fenchlorphos	0.033	0.083	3
fenchlorphos-ethyl	0.096	3.50	36
fenitrothion	0.049	0.62	13
'Chlorthion'	0.17	13.50	79
'Chlorthion'-ethyl	0.036	16.60	461
'Chlorthion'-ethyl ethyl phosphonate	0.066	0.26	4
parathion	0.013	0.85	65
parathion-methyl	0.015	0.23	15
parathion-ethyl phosphonate	0.026	0.096	4
paraoxon	0.037	0.74	20
paraoxon-methyl	0.034	0.28	8
paraoxon-isopropyl	0.31	1.30	4
malathion	0.59	2.30	4
malathion-ethyl	0.29	6.50	22
malaaxon	1.60	12.0	8
phosnichlor	0.028	0.65	23
dichlorvos	0.019	0.037	2
V-Cl-13	0.12	1.90	16

(El Bashir, 1971). The SKA flies were also immune to DDT, a proportion of the population resisted γ -BHC and dieldrin, and although the strain was fully susceptible to kill by pyrethrins it resisted knock-down by this insecticide (Sawicki & Elliott, 1965).

The examination of the cross-tolerance pattern raised more questions than it solved. The pattern of cross-tolerance differed in many respects from that in other organophosphorus-resistant strains (Winteringham & Hewlett, 1964). The SKA flies were extremely resistant to diazinon, the insecticide with which they were selected, but they were also even more resistant to 'Chlorthion'-ethyl with which they had not been in contact. Like other diazinon or parathion selected strains, SKA flies were only slightly resistant to malathion, but resisted malathion-ethyl and malaaxon strongly, and, unlike the Stauffer 'Chlorthion' strain 'Chlorthion'-resistant strain, were almost susceptible to the phosphonates of 'Chlorthion' and parathion (March 1959, 1960).

Piperonyl butoxide, a pyrethrin synergist, now known to inhibit mixed function oxygenases (Casida, 1970) synergised some of the compounds, antagonised others, and had negligible effects of the remainder. The degree of synergism against the susceptible and the resistant flies was similar (Table 2a and b). Another additive, tributylphosphorothionate (TBTP) which inhibits a carboxyesterase (E.C. 3, 1.1.2) (Plapp *et al.*, 1963;

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TABLE 2a

The effect of additives on the toxicity of some organophosphorus insecticides to the SKA strain of houseflies

Insecticide	LD ₅₀ µg/fly			Synergistic/ antagonistic factor	
	Insecticide	piperonyl butoxide	+TBTP	piperonyl butoxide	+TBTP
diazinon	10.47	5.75	5.75	1.88	1.88
fenchlorphos	0.083	0.078	0.074	1.06	1.12
fenchlorphos-ethyl	3.50	4.40	1.40	0.80	2.50
fenitrothion	0.62	0.60	0.63	1.03	0.98
'Chlorthion'	13.50	33.10	1.50	0.41	9.00
'Chlorthion'-ethyl	16.60	*	3.00	*	5.53
'Chlorthion'-ethyl ethyl phosphonate	0.26	0.28	0.30	0.93	0.86
parathion	0.85	0.81	0.71	1.05	1.20
parathion-methyl	0.23	0.25	0.22	0.92	1.05
paraoxon	0.74	0.40	0.66	1.85	1.12
paraoxon-methyl	0.28	0.23	0.31	1.22	0.90
paraoxon-isopropyl	1.30	0.65	1.30	2.00	1.00
malathion	2.30	2.10	1.70	1.10	1.35
malathion-ethyl	6.50	4.60	3.20	1.41	2.03
malaoxon	12.00	2.20	5.20	5.45	2.30
phosnichlor	0.65	0.62	0.56	1.05	1.16
dichlorvos	0.037	—	—	—	—
V-Cl-13	1.90	1.40	1.30	1.36	1.46

* Not measurable

TABLE 2b

The effect of additives on the toxicity of some organophosphorus insecticides to susceptible houseflies

Insecticide	LD ₅₀ µg/fly			Synergistic/ antagonistic factor	
	Insecticide	piperonyl butoxide	+TBTP	piperonyl butoxide	+TBTP
diazinon	0.050	0.040	0.044	1.25	1.14
fenchlorphos	0.033	0.025	0.023	1.32	1.43
fenchlorphos-ethyl	0.096	0.093	0.11	1.03	0.87
fenitrothion	0.049	0.039	0.032	1.26	1.53
'Chlorthion'	0.17	0.21	0.17	0.81	1.0
'Chlorthion'-ethyl	0.036	0.056	0.050	0.64	0.72
'Chlorthion'-ethyl ethyl phosphonate	0.066	0.069	0.071	0.96	0.93
parathion	0.013	0.011	0.015	1.18	0.87
parathion-methyl	0.015	0.017	0.017	0.88	0.88
paraoxon	0.037	0.035	0.045	1.06	0.82
paraoxon-methyl	0.31	0.19	0.26	0.89	0.83
paraoxon-isopropyl	0.31	0.19	0.26	1.63	1.19
malathion	0.59	0.588	0.50	1.02	1.18
malathion-ethyl	0.29	0.19	0.25	1.53	1.16
malaoxon	1.60	0.45	1.20	3.56	1.33
phosnichlor	0.028	0.034	0.032	0.82	0.88
dichlorvos	0.019	—	—	—	—
V-Cl-13	0.12	0.13	0.12	0.92	1.0

Plapp & Tong, 1966) greatly synergised 'Chlorthion' and 'Chlorthion'-ethyl but only against the resistant strains (Table 2a and b).

These and other results (Sawicki & Farnham, 1968b; Sawicki & Green, 1965; Sawicki, 1965; Farnham *et al.*, 1965; Farnham *et al.*, 1966) suggested that resistance in SKA was complex. Biochemical and physiological studies (Gwiazda & Lord, 1967; Farnham *et al.*, 1965) failed to relate the great resistance to any single major metabolic or physiological process, suggesting that it was probably caused by a combination of several minor differences between the susceptible and SKA flies. The problem was therefore to identify

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these mechanisms and determine how they contributed singly and together towards resistance. This could best be done by separating the individual mechanisms of resistance by genetical methods.

Genetics of resistance of strain SKA to organophosphorus insecticides. The location and identification of the resistance mechanisms in the SKA strain was made possible by advances in the study of classical genetics of houseflies by Milani and Hiroyoshi (Milani, 1967). These, and other workers, developed strains of houseflies in which individual pairs of chromosomes could be identified visually because they carried distinct visible morphological mutant markers. Professor R. Milani of the University of Pavia kindly supplied susceptible strains with the following markers for our studies.

ac—curved wings (chromosome I)

ar—aristopedia (i.e. arista modified into a miniature leg (chromosome II))

bwb—brown body (chromosome III)

ocra—ocra eyes (chromosome V)

These visible markers made it possible to recognise the origin of the chromosomes in the progeny of a cross between marked susceptible and unmarked resistant parents. Flies that received one of the two homologous chromosomes of a given pair from the resistant strain were unmarked, i.e. of normal phenotype, whereas flies that received both members of the marked pair from the susceptible strain were marked with the mutant character. When the tolerance of the two phenotypes, i.e. unmarked and marked flies was similar, the investigated chromosome had no non-recessive resistance factors, but when the two phenotypes responded differently to the insecticide the dominant factor of resistance was likely to be located on the same chromosome in the resistant strain as the marker in the susceptible strain (Tsukamoto, 1964).

Crosses between the SKA flies and four susceptible recessive marker strains, each marked on one chromosome, followed by test-crosses with recessive markers and by bioassays of each cross, showed that non-recessive factors of resistance to diazinon were present on chromosomes II and V (Sawicki *et al.*, 1966). These experiments failed however to detect the factor responsible for delaying the penetration of diazinon in the SKA strain which had been detected previously (Farnham *et al.*, 1965).

This preliminary examination (Sawicki & Farnham, 1967a, 1967b, 1968a) was followed by a detailed genetical study of resistance to many insecticides in which each of the autosomes of the SKA strain was isolated in a homozygous condition by inbreeding SKA with the multi-marker susceptible strain *ac*; *ar*; *bwb*; *ocra* SRS (Sawicki & Farnham, 1968b). Isolating each of the five pairs of autosomes without selection with insecticides was very tedious and lengthy, but made it possible to obtain the resistance mechanisms only from the resistant strain. The SKA parent, selected with diazinon, was crossed with the *ac*; *ar*; *bwb*; *ocra* SRS strain. Each of the four triple markers and the quadruple marker segregating in the test-cross progeny ♀ *ac*; *ar*; *bwb*; *ocra* SRS × ♂ F₁ (SKA × *ac*; *ar*; *bwb*; *ocra* SRS) was selfed. The four different combinations were retained for setting up strains. Each of the triple-marker progenies had three marked autosomes derived only from the susceptible parent, the autosome without the fourth marker was derived only from the SKA flies, and the remaining autosome, the fourth unmarked in the susceptible strain, could be inherited from either parent or both. In the quadruple-marker progeny only the fourth autosome unmarked in the susceptible parent was derived from either parent or both. Selection with dieldrin ensured that the fourth autosome was from SKA flies (Sawicki & Farnham, 1968b). Insecticidal pressure at this stage might have speeded the isolation of homozygous strains but could have resulted in the selection of resistance from the genetical background of the susceptible strain.

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TABLE 3
Location of the resistance factors in SKA flies

Chromosomes				
I	II	III	IV	V
	gene <i>a</i> and glutathione transferase	<i>Pen</i>	<i>Dld</i>	<i>Ses</i>
'Chlorthion' c. × 2	parathion × 15 paraoxon × 3.6 parathion-methyl × 7 paraoxon-methyl × 2.4 malathion-ethyl × 4 malaaxon-ethyl × 6.5 malathion × 1.4 malaaxon × 6 'Chlorthion'-ethyl × 48 'Chloroxon'-ethyl × 14 'Chlorthion' × 14 'Chloroxon' × 12 diazinon × 13 diazoxon × 8 <i>Deh</i> DDT × 1000>	diazinon × < 2 'Chlorthion' × < 2 dieldrin × < 2 DDT × < 2 tributyl tin acetate × 12	dieldrin × 700	diazinon × 9 diazoxon × 4.6 malaaxon-ethyl × 3 malaaxon × < 2 DDT × 10 methoxychlor × 20

Each of the five strains with one pair of chromosomes from SKAs was tested with 14 insecticides to locate and measure the individual resistance factors by comparing the response with that of the susceptible *ac*; *ar*; *bwb*; *ocra* SRS parent. Table 3 shows that

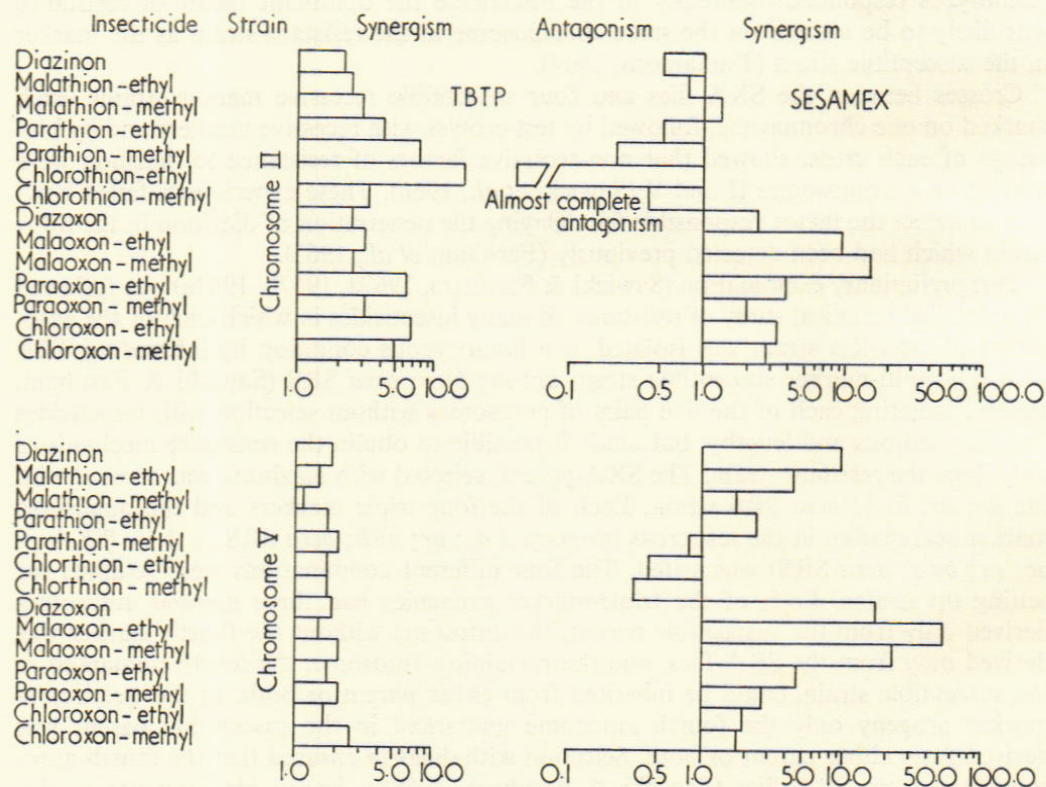


FIG. 1a. Synergism and antagonism of the susceptible *ac*; *ar*; *bwb*; *ocra* SRS and the resistant SKA strains to 14 organophosphorus insecticides after pre-treatment with TBTP and sesamex (synergism and antagonism calculated at LD₅₀).

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moderate resistance was found only on chromosomes II and V and that neither factor conferred much resistance to OP insecticides.

Pretreatment with sesamex, which inhibits mixed function oxygenases, or TBTP (tributyl phosphorotrithioate) which inhibits carboxy esterases, produced different responses to the insecticides in strains with chromosome II or V derived from SKA (Fig. 1a and b). TBTP greatly synergised 'Chlorthion'-ethyl and parathion-methyl and decreased resistance to seven other compounds in the strain with chromosome II, but had little effect on the response of flies with each of the other pairs of autosomes from strain SKA or on strain SKA itself.

Pre-treatment with sesamex gave both antagonism (when kill after treatment was less than kill by the insecticide alone) and synergism (when pre-treatment increased kill). Antagonism, which occurred only with the thionates, especially parathion-methyl and 'Chlorthion'-ethyl, was most evident in flies with resistance mechanisms on chromosome II (Fig. 1). Synergism, which in most substrains of SKA was confined to phosphates, was greater in the strain with SKA's chromosome V and in this strain sesamex synergised diazinon very much. The SKA strain itself, which has the resistance mechanism(s) on chromosome II, antagonised by, and on chromosome V synergised by sesamex, is least affected by pre-treatment with this compound.

These experiments suggested that the mechanism on chromosome V was most likely to be detoxication by a mixed function oxygenase. They failed to reveal the number or nature of the mechanisms of resistance on chromosome II and this was later done biochemically.

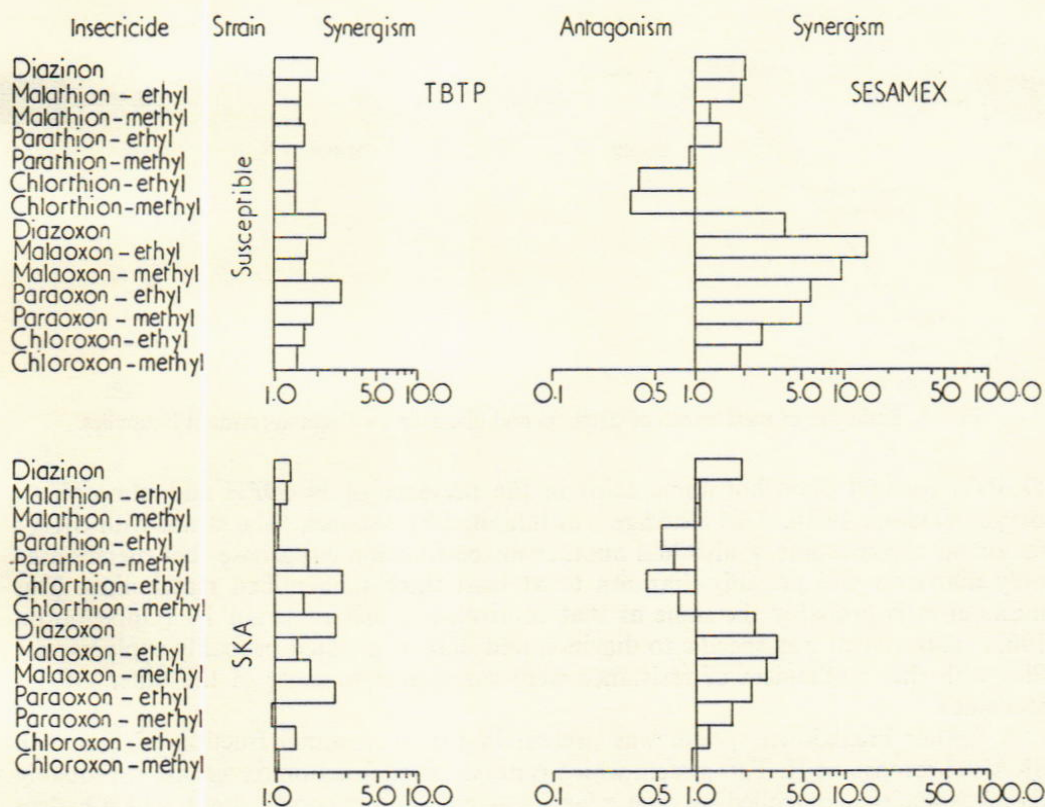


Fig. 1b. Synergism and antagonism of strains in SKA's chromosomes II and V to 14 organophosphorus insecticides after pre-treatment with TBTP and sesamex (synergism and antagonism calculated at LD₅₀).

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Identification of the mechanisms of resistance on chromosomes II and V. Sub-cellular fractions of houseflies susceptible and resistant to diazinon were examined to characterise *in vitro* the mechanism of resistance to diazinon, parathion and diazoxon controlled by genes on chromosome II and V (Lewis, 1969; Lewis & Sawicki, 1971).

The supernatant fluid from centrifuging whole-fly homogenates in 0.15M phosphate buffer (pH 7.3) at 13 000 g for ten minutes was either used as an enzyme source or re-centrifuged at 100 000 g for one hour to give microsomal (precipitate) and soluble (fluid) fractions. Each sub-cellular fraction was incubated with radio-active insecticide in the presence or absence of NADPH or reduced glutathione (GSH). Metabolites were examined, and identified by two-dimensional chromatography.

The microsomal and soluble fractions contained different insecticide metabolising systems with different co-factor requirements (Fig. 1). In all strains the microsome-containing fraction contained a mixed function oxygenase that converted the phosphorothionate insecticides to their oxygen analogues and cleaved diazinon and parathion to

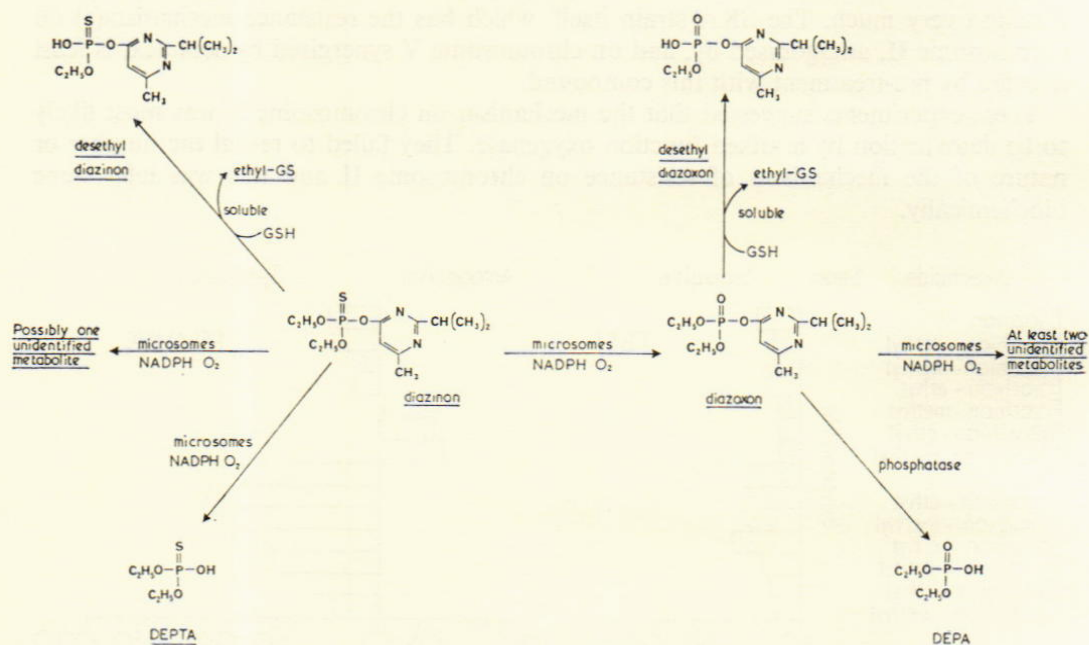


FIG. 2. Pathways of metabolism of diazinon and diazoxon in diazinon-resistant houseflies.

DEPTA (diethyl phosphorothioic acid) in the presence of NADPH and atmospheric oxygen (Dahm, 1970). This cleavage was inhibited by sesamex. The strain with SKA's factor on chromosome V also had another mixed-function oxygenase that metabolised only diazoxon and possibly diazinon to at least three unidentified metabolites. This mechanism is probably the same as that controlled by *md* in strain F_c (Oppenoorth, 1967). This system was specific to diazinon and diazoxon which probably explains why flies with this mechanism of resistance were susceptible to most of the other OP insecticides.

A further breakdown system was present in the microsomal fraction of flies with SKA's chromosome II. This system which is not a mixed function oxygenase is probably the phosphatase, controlled by gene *a* (van Asperen & Oppenoorth, 1960) which hydrolyses the oxygen analogues of OP insecticides giving DEPA (diethyl phosphoric acid). Flies with resistance on chromosome II had a further breakdown system that was GSH

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dependent (an alkyl-transferase). This enzyme which desethylated diazinon, diazoxon and parathion with the concomitant formation of S-ethyl glutathione may explain why SKA flies are more resistant to the diethoxy than the dimethoxy insecticides (Busvine, 1959).

The present of two systems unaffected by sesamex, i.e. phosphatase and GSH transferase, explains why sesamex acts as an antagonist to phosphorothioates in strains with SKA's chromosome II. Sesamex inhibits or delays the activation of phosphorothioates to phosphates without impairing their detoxication by either GSH transferase or phosphatase.

The metabolism of 'Chlorthion'-ethyl and diazinon in flies with SKA's chromosome II differ. Both compounds are probably modified by a soluble, glutathione requiring mechanism, but the breakdown products are not strictly analogous, because relatively less desethyl 'Chlorthion' and more of an unidentified metabolite, possibly an amino acid derivative, are found. This metabolite is not found after pre-treatment with TBTP (Lewis & Lord, 1969).

Penetration delaying factor. The penetration delaying factor (Pen) was first suspected when it was noticed injection greatly reduced resistance to diazinon. Later it was shown that after treatment with dieldrin flies of the strain with chromosome III derived from strain SKA showed symptoms of poisoning much later than flies of other strains derived from strain SKA, although the dose to kill was no larger than for the other strains (Sawicki & Farnham, 1967b). This delayed knock-down was also noticed after treatment with DDT and diazinon, although again there was little difference in susceptibility—the factor gave resistance of less than $\times 2$. The cause was traced to delayed penetration of insecticides through the cuticle and the factor was therefore given the name *Pen*.

The isolation of *Pen* enabled some of its properties to be determined without the complication of interactions with other resistance mechanisms (Sawicki & Lord, 1970). *Pen* delays but does not prevent penetration through the cuticle, and its effect is greatest for small doses and with non-polar insecticides. It is most effective at delaying the entry of dieldrin and DDT, much less so against diazinon, parathion and 'Chlorthion'-ethyl and is least effective for diazoxon, the most polar of the insecticides tested. It is already present in two hour-old adults, i.e. before the cuticle has fully hardened and is more effective in delaying penetration in females than males. *Pen* alone confers weak resistance to most insecticides because the slower entry enables the insect to detoxify more insecticide than flies with normal penetration. Greatest resistance ($\times 12$) occurs with tributyl tin which is very rapidly hydrolysed by houseflies (Hoyer & Plapp, 1968). It acts as an intensifier of resistance in the presence of major mechanisms of resistance (Plapp & Hoyer, 1968). The nature of the mechanism is not known.

Interaction between mechanisms of resistance. Biochemical and genetical work showed that singly none of the individual mechanisms responsible for the strong resistance of strain SKA to diazinon gives much resistance. Therefore strong resistance is probably caused by interactions between the resistance factors when together. To test this, the parent strain (strain SKA) was sequentially reconstituted by the re-synthesis of multi-resistance from strains with the single SKA chromosomes. All three possible combinations of pairs of factors of resistance were inbred and tested for their combined effect on resistance. These combinations were:

SKA's chromosome	II and III
	II and V
	III and V

Figures 3a and b and Table 5 summarise the results.

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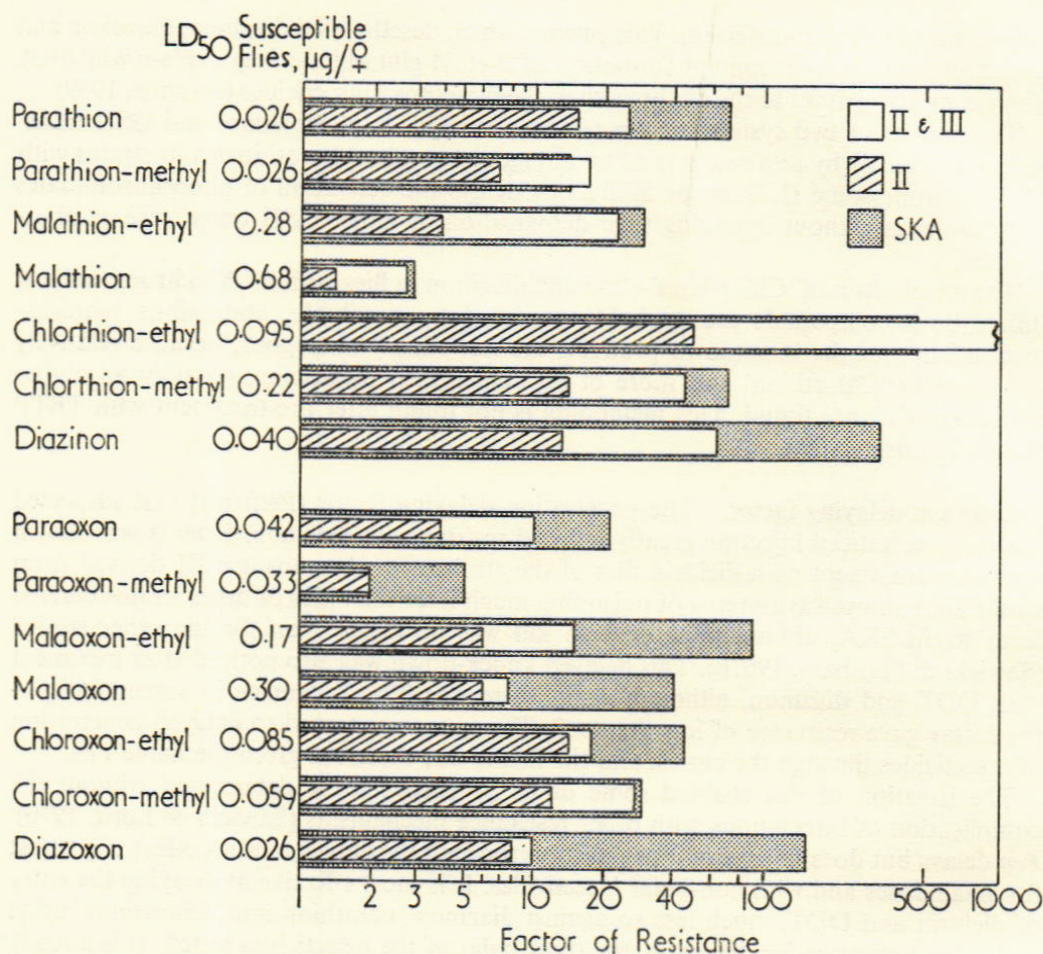


Fig. 3a. Cross-resistance spectrum of flies with SKA's chromosomes II, II and III, and strain SKA.

(i) *Pen* (chromosome III) was successfully bred into a strain with SKA's chromosome II using the method of substituting the chromosome with visible mutant markers lacking factors of resistance by the unmarked chromosome with the resistance factors (Sawicki, 1970). It was necessary to select with insecticides to obtain the other combinations of pairs of chromosomes because the breeding method used for chromosomes II and III failed. Too much inbreeding and crossing by single pairs made flies sterile.

Bio-assays of flies of the reconstituted strain (SKA's chromosomes II and III) showed convincingly that most of the resistance of the SKA strain to thionates was caused by delayed penetration increasing the resistance of flies with resistance mechanisms on chromosome II (Sawicki, 1970). This combined activity produced a resistance identical to and hence fully accounting for the resistance of strain SKA to parathion-methyl, malathion, chloroxon and 'Chlorthion'-ethyl and for most of the resistance against 'Chlorthion' and malathion-ethyl (Fig. 2). Delayed penetration also increased resistance to the corresponding phosphates (except 'Chloroxon') but less than for the thionates, partly because phosphates penetrate faster.

(ii) A completely different cross-resistance spectrum was shown by flies with SKA's chromosomes II and V (Fig. 3) (Sawicki, 1972, in the press). Introducing the microsomal detoxifying mechanism on chromosome V which confers resistance only against a few of the organophosphorus insecticides, into a strain with the resistance mechanisms on

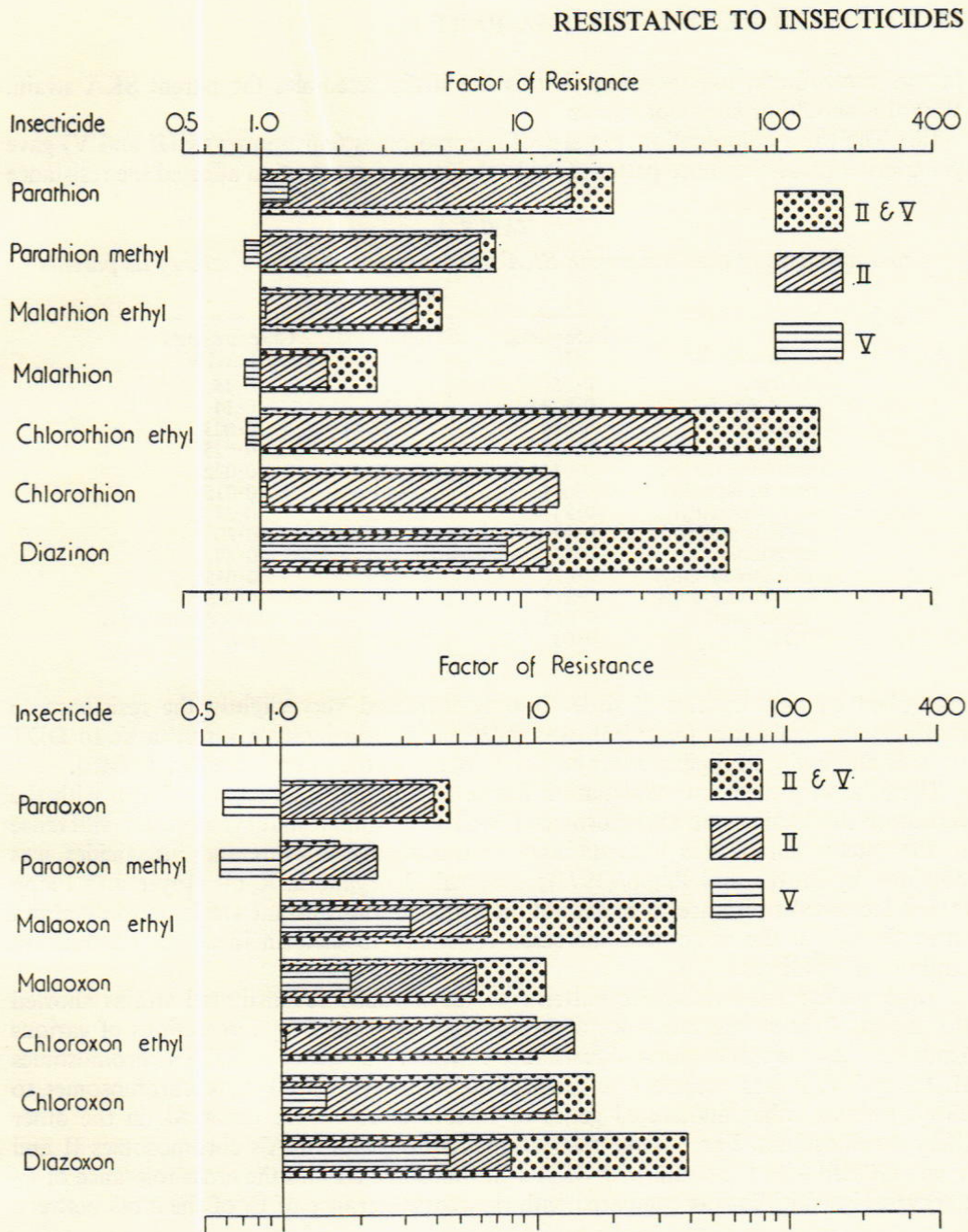


FIG. 3b. Cross-resistance spectra of flies with SKA's chromosomes II, V, and II and V.

chromosome II greatly increased resistance, but almost exclusively to insecticides against which both factors of resistance were effective. However it also very slightly increased resistance against most of the other insecticides. The greatly increased resistance to diazinon, diazoxon, malaoxon and malaoxon-ethyl approximated the product of the resistances conferred by each mechanism singly, suggesting that the mechanisms of resistance act independently rather than interact. The reasons for a large increase in resistance to 'Chlorthion'-ethyl in flies with SKA's chromosomes II and V are not known. The marked synergism or antagonism with sesamex and tributyl phosphorotrithionate which occurs in the strain with single resistance mechanisms disappears when the two

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factors are brought together, and in this the strain resembles the parent SKA strain. Why this should be so is not known.

(iii) The third combination of pairs of chromosomes (chromosomes III and V) gave yet another cross-resistance pattern (Table 4). Delayed penetration affected the resistance

TABLE 4
Cross tolerance of the strains with SKA's chromosomes III and V and of its parents

Insecticide	LD ₅₀ µg/♀		
	Chromosome III	Chromosome V	Chromosomes III and V
diazinon	0.061	0.37	0.45
diazoxon	0.029	0.12	0.14
parathion	0.026	0.032	0.023
paraoxon	0.050	0.026	0.025
parathion-methyl	0.044	0.028	0.022
paraoxon-methyl	0.038	0.019	0.015
malathion-ethyl	0.24	0.28	0.27
malaoxon-ethyl	0.24	0.57	0.80
malathion	0.37	0.48	0.49
'Chlorthion'-ethyl	0.078	0.082	0.085
'Chloroxon'-ethyl	0.095	0.084	0.086
'Chloroxon'	0.065	0.092	0.068
DDT	0.60	2.50	5.6

controlled by chromosome V little. It only increased very slightly the resistance to diazinon and malaoxon (*c.* ×1.5), was most effective in increasing resistance to DDT (*c.* ×6) and had a negligible effect on the response to the other insecticides tested.

There is, at present, no explanation for these different interactions of *Pen* with the resistance mechanisms on chromosomes II and V. A similar and even greater difference in the interaction of *Pen* with different resistance mechanisms and insecticides was obtained by Hoyer and Plapp (1971); *Pen*, called organotin-R by Hoyer and Plapp (1968) increased resistance to malathion in a malathion-resistant strain by only about three times, but the same gene increased resistance to dieldrin in a dieldrin-resistant strain over 100 times.

Although the cross-resistance patterns of the partially reconstituted strains showed that much of the strong resistance of strain SKA was caused by interactions of various kinds between the mechanisms of resistance controlled by genes on SKA's chromosomes II, III and V, it was necessary to reconstitute a strain with all three chromosomes to check whether other undetected genes or factors of resistance occurred on the other SKA chromosomes. For this the reconstituted strain with SKA's chromosomes II and V was crossed with the strain with SKA's chromosome III and the cross tolerance of F₁ to several insecticides was compared with the cross tolerance of F₁ of the cross between

TABLE 5
Comparison between the cross-tolerance of F₁ hybrids of cross SKA's II and V × SKA III (in which chromosomes II, III and V isolated from strain SKA are combined and are heterozygous) and F₁ hybrids of cross *ac; ar; bwb; ocra* SRS × SKA

Insecticides	LD ₅₀ µg/♀	
	F ₁ (II and V × III)	F ₁ (<i>ac; ar; bwb; ocra</i> SRS × SKA)
diazinon	1.25	1.60
parathion	0.42	0.40
malaoxon-ethyl	4.0	5.8
'Chlorthion'-ethyl	5.4	5.0

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SKA and *ac; ar; bwb; ocr* SRS, the susceptible parent. The two F₁ progenies gave similar results (Table 5) indicating that (i) the two F₁s had the same non-recessive genes for resistance; (ii) strain SKA was unlikely to have additional non-recessive genes on the other chromosomes; (iii) that resistance in strain SKA could be satisfactorily explained in terms of interactions between the known mechanisms of resistance.

The complete reconstitution of the SKA strain from its isolated chromosomes was not attempted. The additional information was unlikely to justify the enormous amount of work to fully reconstitute the SKA strain.

Biochemistry and genetics of resistance to DDT. The SKA strain not only resisted OP, but like most other OP resistant strains it was also very resistant to DDT, even though it had not been either selected or in contact with this insecticide at any stage. SKA probably inherited DDT resistance from its parents which had become resistant to this insecticide before it had been replaced in the field by diazinon.

SKA flies differed biochemically in several ways from strains susceptible to or selected with and resistant to DDT (El Bashir & Lord, 1965; El Bashir, 1967). In such resistant strains, resistance is caused by DDT-dehydrochlorinase controlled by gene *Deh* on chromosome II (Oppenoorth, 1964). However in SKA, DDT penetrated slower and accumulated less than in other strains, and the main metabolite (DDE), present in large amounts in other DDT-resistant strains, was readily metabolised. Pre-treatment with WARF-anti-resistant (*N,N*-di-*n*-butyl-*p*-chlorobenzene sulphonamide) or FDMC (bis-*(p*-chlorophenyl) trifluoromethyl carbinol), both DDT-dehydrochlorinase inhibitors, or with sesamex increased kill by DDT little or not at all. This suggested that DDT resistance in SKA flies was caused by neither DDT-dehydrochlorinase nor microsomal detoxication. However, genetical analysis of resistance to DDT in strain SKA showed that both factors were present.

Bioassays of flies with individual chromosomes derived from strain SKA showed that DDT-resistance mechanisms were associated with SKA's chromosomes II or V, and that delayed penetration on chromosome III decreased kill very slightly. The factor on chromosome II homozygous in only 20% of the population, was inhibited by FDMC and was therefore likely to be DDT-dehydrochlorinase. The factor on chromosome V, which conferred only *c.* ×10 resistance, was completely inhibited by pre-treatment with sesamex and was probably identical to *Ses*, the microsomal detoxication mechanism, identical to *DDTmd* of strain Fc (Oppenoorth & Houx, 1968). This factor was shown by us to interact with *Pen* to give greater resistance to DDT. The very strong resistance conferred by DDT-dehydrochlorinase alone is likely to be stronger still in the presence of *Pen* (Hoyer & Plapp, 1971). Interactions between *Ses* and *Pen*, and *Deh* and *Pen* probably explain why inhibition of either by the synergists increased kill little and only pre-treatment with both synergists followed by treatment with DDT eliminated resistance completely.

Genetics of resistance to dieldrin. Resistance to dieldrin in strain SKA is controlled by a gene on chromosome IV probably identical or an allele of *Dld* of other dieldrin-resistant strains (Georghiou *et al.*, 1963; Oppenoorth & Nasrat, 1966). This gene confers immunity to topically applied dieldrin in acetone during the first 24 hours but increasing kill during the next 72 hours decreases the resistance factor to *c.* ×700. The proportion of SKA flies with this gene has decreased steadily from 25% in 1964 to 10% in 1967, and is now so rare that A. W. Farnham recently (August 1972) failed to detect it amongst 675 female flies examined. This is because resistance to dieldrin, which developed as a result of application of chlordane in the field, was independent of other mechanisms of

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resistance and was unaffected by selection with diazinon. In spite of this it persisted in this population for about 20 years.

Dieldrin resistance was probably derived from the parents of SKA which had been treated in the field with chlordane.

Conclusions

Much is now known about reasons for the strong resistance of the SKA strain to many insecticides, and recent work on other strains has confirmed our findings (Georghiou, 1971). The multiple nature of resistance (Tsukamoto, 1969) shows clearly the complexity of the problem, and explains why there are still no means of overcoming resistance.

The weak individual mechanisms of resistance in SKA flies interact to give strong resistance. The diversity of these mechanisms precludes the use of the usual synergists to overcome resistance because their interactions with some of the factors may actually increase resistance. Interaction is complex because it depends not only on the mechanisms involved but also the insecticides, and at present neither the likelihood nor degree of resistance are predictable. Control is difficult and has to rely on new insecticides against which the insects have not yet developed resistance. The effectiveness of these insecticides is usually short lived because multiple-resistant strains develop new resistance mechanisms very rapidly. Why this should be so, and how resistance develops, is not known.

REFERENCES

- BUSVINE, J. R. (1959) Patterns of insecticide resistance to organophosphorus compounds in strains of houseflies from various sources. *Entomologia experimentalis et applicata* **2**, 58–67.
- CASIDA, J. E. (1970) Mixed-function oxidase involvement in the biochemistry of insecticide synergists. *Journal of Agricultural and Food Chemistry* **18**, 753–772.
- DAHM, P. A. (1970) Some aspects of the metabolism of parathion and diazinon. In: *Biochemical Toxicology of Insecticides*. Eds. R. D. O'Brien & I. Yamamoto, New York: Academic Press.
- EL BASHIR, EL S. (1967) Causes of resistance to DDT in a diazinon-selected and a DDT-selected strain of houseflies. *Entomologia experimentalis et applicata* **10**, 111–126.
- EL BASHIR, EL S. (1971) Resistance to organophosphorus insecticides in the SKA strain of houseflies (*Musca domestica*). *Entomologia experimentalis et applicata* **14**, 365–376.
- EL BASHIR, EL S. & LORD, K. A. (1965) DDT tolerance in diazinon-selected and DDT-selected strains of houseflies. *Chemistry and Industry* 1598–1599.
- FARNHAM, A. W., GREGORY, G. E. & SAWICKI, R. M. (1966) Bioassay and histochemical studies of the poisoning and recovery of houseflies (*Musca domestica* L.) treated with diazinon and diazoxon. *Bulletin of Entomological Research* **57**, 107–116.
- FARNHAM, A. W., LORD, K. A. & SAWICKI, R. M. (1965) Study of some of the mechanisms connected with resistance to diazinon and diazoxon in a diazinon-resistant strain of houseflies. *Journal of Insect Physiology* **11**, 1475–1488.
- GEORGHIOU, G. P. (1971) Isolation, characterization and re-synthesis of insecticide resistance factors in the housefly, *Musca domestica*. *Proceedings, 2nd International Congress of Pesticide Chemistry* **2**, 77–94.
- GEORGHIOU, J. P., MARCH, R. B. & PRINTY, G. E. (1963) A study of the genetics of dieldrin-resistance in the housefly (*Musca domestica* L.). *Bulletin of the World Health Organization* **29**, 155–165.
- GWIAZDA, M. & LORD, K. A. (1967) Factors affecting the toxicity of diazinon to *Musca domestica* L. *Annals of Applied Biology* **59**, 221–232.
- HOYER, R. F. & PLAPP, JR., F. W. (1968) Insecticide resistance in the housefly: identification of a gene that confers resistance to organotin insecticides and acts as an intensifier of parathion resistance. *Journal of Economic Entomology* **61**, 1269–1276.
- HOYER, R. F. & PLAPP, JR., F. W. (1971) Insecticide resistance in the housefly, effect of a modifier gene in combination with major genes which confer resistance. *Journal of Economic Entomology* **64**, 1051–1055.
- LEWIS, J. B. (1969) Detoxification of diazinon by sub-cellular fractions of diazinon resistance and susceptible houseflies. *Nature, London* **224**, 917–918.
- LEWIS, J. B. & LORD, K. A. (1969) Metabolism of some organophosphorus insecticides by strains of housefly. *Proceedings of the 5th British Insecticides and Fungicide Conference* 455–471.
- LEWIS, J. B. & SAWICKI, R. M. (1971) Characterization of the resistance mechanisms to diazinon, parathion and diazoxon in the organophosphorus resistant SKA strain of houseflies (*Musca domestica* L.). *Pesticide Biochemistry and Physiology* **1**, 275–285.

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- MARCH, R. B. (1959) Resistance to organophosphorus insecticides. *Miscellaneous publications of the Entomological Society of America* 1, 13-19.
- MARCH, R. B. (1960) Biochemical aspects of organophosphorus resistance. *Miscellaneous publications of the Entomological Society of America* 2, 139-144.
- MILANI, R. (1967) The genetics of *Musca domestica* and other Muscoid flies. In: *Genetics of Insect Vectors of Disease*. Ed. J. W. Wright & R. Pal. Amsterdam: Elsevier Publishing Co., 794 pp.
- OPPENORTH, F. J. (1964) Some cases of resistance caused by the alteration of enzymes. *12th International Congress of Entomology* London, 240-242.
- OPPENORTH, F. J. (1967) Two types of sesamex-susceptible resistance in the housefly. *Entomologia experimentalis et applicata* 10, 75-86.
- OPPENORTH, F. J. & HOUX, N. W. H. (1968) DDT-resistance in the housefly caused by microsomal degradation. *Entomologia experimentalis et applicata* 11, 81-93.
- OPPENORTH, F. J. & NASRAT, G. E. (1966) Genetics of dieldrin and gamma-BHC (lindane) resistance in the housefly (*Musca domestica*). *Entomologia experimentalis et applicata* 9, 223-231.
- PLAPP, JR. F. W., BIGLEY, W. S., CHAPMAN, G. A. & EDDY, G. W. (1963) Synergism of malathion against resistant houseflies and mosquitoes. *Journal of Economic Entomology* 56, 643-649.
- PLAPP, JR. F. W. & HOYER, R. F. (1968) Insecticide resistance in the housefly: decreased rate of adsorption as a mechanism of action of a gene that acts as an intensifier of resistance. *Journal of Economic Entomology* 61, 1298-1303.
- PLAPP, JR. F. W. & TONG, H. H. C. (1966) Synergism of malathion and parathion against resistant insects: phosphorus esters with synergistic properties. *Journal of Economic Entomology* 59, 11-15.
- SAWICKI, R. M. (1965) Similarity of response to diazinon, dieldrin and pyrethrum extract in allatectomised and normal houseflies (*Musca domestica* L.). *Bulletin of Entomological Research* 55, 727-732.
- SAWICKI, R. M. (1970) Interaction between the factor delaying penetration of insecticides and the desethylation mechanism of resistance to organophosphorus-resistant houseflies. *Pesticide Science* 1, 84-87.
- SAWICKI, R. M. (1973) Resynthesis of multiple resistance to organophosphorus insecticides from strains with factors of resistance isolated from the SKA strain of houseflies. *Pesticide Science*, 4 (In the press.)
- SAWICKI, R. M. & ELLIOTT, M. (1965) Relative toxicity of pyrethrin I and pyrethrin II against four strains of houseflies. *Journal of the Science of Food and Agriculture* 16, 85-89.
- SAWICKI, R. M. & FARNHAM, A. W. (1964) A dipping technique for selecting houseflies for resistance to insecticides conferred with a measured-drop technique. *Bulletin of Entomological Research* 55, 541-546.
- SAWICKI, R. M. & FARNHAM, A. W. (1967a) Genetics of resistance to insecticides of the SKA strain of *Musca domestica*. I. Location of the main factors responsible for the maintenance of high DDT-resistance in diazinon-selected SKA flies. *Entomologia experimentalis et applicata* 10, 253-262.
- SAWICKI, R. M. & FARNHAM, A. W. (1967b) Genetics of resistance to insecticides of the SKA strain of *Musca domestica*. II. Isolation of the dominant factors of resistance to diazinon. *Entomologia experimentalis et applicata* 10, 363-376.
- SAWICKI, R. M. & FARNHAM, A. W. (1968a) Genetics of resistance to insecticides of the SKA strain of *Musca domestica*. III. Location and isolation of the factors of resistance to dieldrin. *Entomologia experimentalis et applicata* 11, 133-142.
- SAWICKI, R. M. & FARNHAM, A. W. (1968b) Examination of the isolated autosomes of the SKA strain of houseflies (*Musca domestica* L.) for resistance to several insecticides with and without pretreatment with sesamex and TBTP. *Bulletin of Entomological Research* 59, 409-421.
- SAWICKI, R. M. & GREEN, G. (1965) Changes in the susceptibility of normal and resistant houseflies (*Musca domestica* L.) to diazinon with age. *Bulletin of Entomological Research* 55, 715-725.
- SAWICKI, R. M., FRANCO, M. G. & MILANI, R. (1966) Genetic analysis of the non-recessive factors of resistance to diazinon in the SKA strain of the housefly (*Musca domestica* L.). *Bulletin of the World Health Organization* 35, 893-903.
- SAWICKI, R. M. & LORD, K. A. (1970) Some properties of a mechanism delaying penetration of insecticides into houseflies. *Pesticide Science* 1, 213-217.
- TSUKAMOTO, M. (1964) Methods for the linkage-group determination of insecticide-resistance factors in the housefly. *Botyu-Kagaku* 29, 51-59.
- TSUKAMOTO, M. (1969) Biochemical genetics of resistance in the housefly. *Residue Reviews* 25, 289-314.
- VAN ASPEREN, K. & OPPENORTH, F. J. (1960) The interaction between organophosphorus insecticides and esterases in homogenates of organophosphorus susceptible and resistant houseflies. *Entomologia experimentalis et applicata* 3, 68.
- WINTERINGHAM, F. P. W. & HEWLETT, P. S. (1964) Insect cross resistance phenomena, their practical and fundamental implications. *Chemistry and Industry* 35, 1512-1518.