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INVESTIGATIONS OF FRUIT FLIES IN HAWAII
(Formerly Oriental Fruit Fly Investigations.)

QUARTERLY REPORT

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WORK PROJECT I-c-6. Physiology of the Oriental Fruit Fly - C. C. Roan, Project Leader.

SUMMARY

The *in vivo* actions of eight selected compounds on three species of flies were investigated. The results of these determinations when compared with *in vitro* data suggest differences in the biochemistry of these flies.

The human plasma and red blood cell cholinesterase activities were measured by the micro-colorimetric method.

Four strains of D. dorsalis are being selected for parathion and DDT resistance. Topical treatment of the parathion resistance strain showed no increased tolerance to parathion in the 4th generation.

The mass production of D. dorsalis and C. capitata has been satisfactory. These cultures have been maintained at a half a million level which is adequate to supply the various projects and also to accommodate any sudden needs for these species. The demands for melon flies are gradually being met. With the major harvesting of melon host fruits in the field, it was necessary to concentrate on the laboratory culturing of the flies for it was difficult to supplement the stock cultures with trapped wild flies.

Line Project I-c-6-2. Reaction of the Oriental Fruit Fly to Light, Temperature and Chemical Stimuli.

I. The *in vivo* Action of Selected Anticholinesterase Compounds on the Oriental Fruit Fly, the Mediterranean Fruit Fly and the Melon Fly. (C. C. Roan and S. Wade)

The toxic effects of eight representative compounds on the three species of flies were investigated. The compounds were selected to represent the organic phosphates, including an oxygen and a sulfur compound, and the carbamates with typical structural variations. These materials are listed in table 1. The dosage mortality data were determined by topical applications of acetone solutions of the selected toxicants to the venter of the thorax of flies of uniform age. The applications were made by the use of the micropipette described in the previous report. The per cent mortality at 24 hours was plotted against the dosage on log-probit paper, and the LD-50's determined directly from such plots.

There is a considerable variation in the toxic values of these selected compounds as shown in table 1. Although the *in vitro* action of these compounds against the brain ChE of the three species of flies were quite similar and indicated a similarity in the nature of the enzymes, the data in table 1 show a species differentiation towards toxicity that could not be predicated from the *in vitro* data given in the last quarterly report.

The effect of paraoxon on these species was more toxic than the sulfur analogue of paraoxon--parathion as also indicated by the *in vitro* tests.

The carbamate compound G-22008 is less than half as effective *in vitro* as paraoxon. In general, the differences in *in vivo* toxicity between two materials is of the same order of magnitude with the exception of *C. capitata*. Here the toxicity of G-22008 is only 1/20 of that of paraoxon.

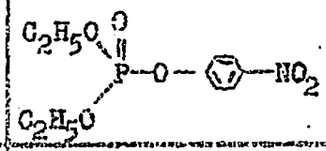
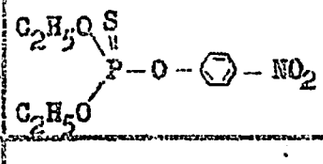
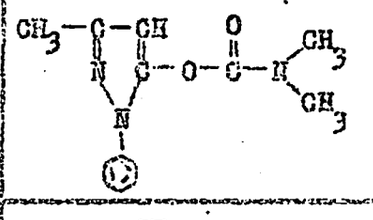
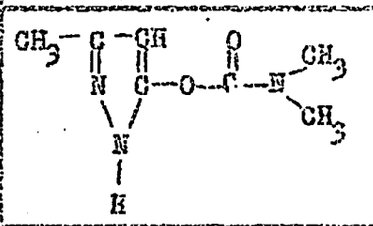
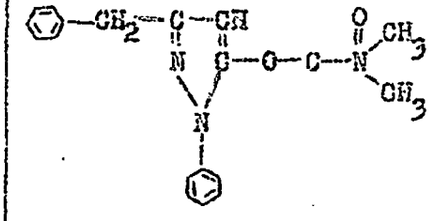
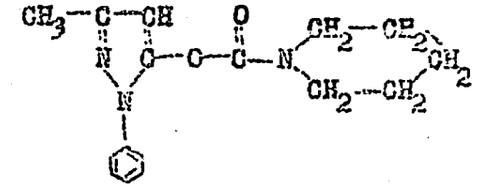
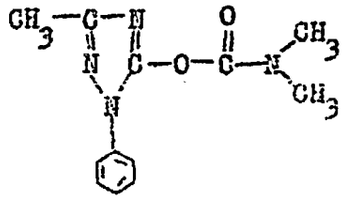
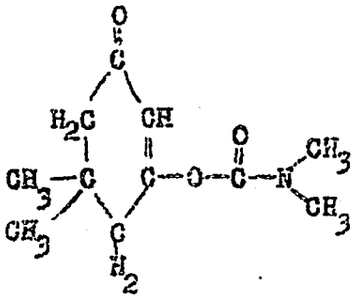
The G-compounds represent structure variations of G-22008, the parent carbamate compound. According to the *in vitro* determination, ten to twenty times more or equivalent amount of these compounds was required to obtain a comparable inhibition to that produced by G-22008. In *in vivo* tests, the toxicity varied anywhere from 0.6 to 1/20.

G-22870 is less than 1/20 as toxic to *D. dorsalis* *in vitro* as G-22008, but *in vivo* is more than twice as toxic. This same relationship holds for all three species. This greater *in vivo* toxicity possibly is due to the lack of a phenyl group on the pyrazolyl ring of G-22870, which would permit more rapid penetration to the site of action.

G-23012, which is the same as G-22008 with an added phenyl ring, is less toxic *in vivo* to *D. dorsalis* and *D. cucurbitae*, but curiously enough is more toxic to *C. capitata* than G-22008.

G-23328, in which the nitrogen atom of the carbamate part of G-22008 is contained in a heterocyclic, piperidine ring, is much less toxic *in vivo* than G-22008 to all three species, although the differences in *in vitro* toxicity is only slight.

Table 1. The in vitro and in vivo effects of D. dorsalis, D. cucurbitae and C. capitata by certain insecticides.

	COMPOUNDS	D. dorsalis		D. cucurbitae		C. capitata	
		IN_{50} 1/	LD_{50} 2/	IN_{50}	LD_{50}	IN_{50}	LD_{50}
Paraoxon		$9X10^{-9}$	0.37	$8.8X10^{-9}$	0.53	$1X10^{-8}$	0.23
Parathion		$2.3X10^{-7}$	1.14	$8.6X10^{-8}$	0.88	$1X10^{-7}$	1.00
G-22008		$2X10^{-6}$	1.31	$7.8X10^{-8}$	1.20	$2.8X10^{-8}$	4.60
G-22870		$5X10^{-7}$	0.6	$5.4X10^{-7}$	0.8	$7.8X10^{-7}$	1.97
G-23012		$3.7X10^{-8}$	96	$4X10^{-9}$	14	$4.2X10^{-8}$	1.81
G-23328		$9.2X10^{-8}$	200	$5.4X10^{-8}$	144	$9X10^{-8}$	30
G-23162		$9.2X10^{-6}$	1720	$8.2X10^{-6}$	256	$8.4X10^{-6}$	96
G-19258		$5X10^{-7}$	117	$6.5X10^{-7}$	128	$5.6X10^{-7}$	92

1/ IN_{50}= Final molar concentration

2/ LD_{50}= Mg/gram of body weight.

Table 2. Larval and adult selection for parathion resistance.

	STRAIN I						STRAIN II			
	Larval Stage		Adult Stage				Larval Stage		Adult Stage not treated	
Generation	p.p.m. parathion in medium	Larval mortality	Dosage	Adult mortality	LD 50 µg/gm of body weight	Control	p.p.m. parathion in medium	Larval mortality	LD 50 µg/gm of body weight	Control
F_1	0.02	73%	Topical application .08 µg/fly	90%			0.02	73%		
F_2	0.02	75%	Residual 0.5 mg/100	80%			0.02	75%		
F_3	0.02	77%	0.5 mg/100	77%			0.02	77%		
F_4	0.02	37%	0.5 mg/100	47%			0.02	37%		
F_5	0.028	68%	1.5 mg/100	83%	0.4	0.47	0.028	72%	0.45	0.47

G-23162, replacing a triazolyl ring for the pyrazolyl ring of G-22008 reduces both the *in vitro* and *in vivo* toxicity by factor of between 100 and 1000 times.

G-19258 is related to the parent G-22008 only in the dimethyl carbamate part of the molecule. This alternation reduces the *in vivo* toxicity to all three species by factor of about 100.

Each of these carbamate compounds gave a more or less similar degree of *in vitro* inhibition for the three species of flies. Among the different compounds tested the widest range in inhibition was by a factor of 20. However, the toxicity effect of these compounds showed pronounced differences, even between species. These differences suggest differences in the biochemistry involved, such as a more toxic metabolite forming or more rapid or effective detoxification mechanisms, and undoubtedly variations in the permeability of these compounds through the cuticle.

Human Plasma and Red Blood Cell Cholinesterase Activities.

The plasma and red blood cell cholinesterase (ChE) activities of individuals engaged in field testing of insecticides were investigated as a possible protective measure from over-exposure to the organic phosphates. The procedure used was the microcolorimetric method described by Metcalf.1/ The results obtained from six samples of male blood indicated that the plasma ChE activity varied from 0.1 to 0.36 millimoles of acetylcholine hydrolysed per ml. plasma per hour at 37° C. which was within the normal range reported by Metcalf. The red blood cell was lysed and one ml. of this material hydrolysed anywhere from 0.02 to 0.14 millimoles of acetylcholine per hour at 37° C.

II. The Potential Resistance of the Oriental Fruit Fly to Parathion. (C. C. Roan and S. Maeda)

There was an indication of increased tolerance to parathion in the fourth generation. Concentrations of parathion were selected to give an overall 70-90% larval and adult mortalities. The initial dosages used in the larval and adult treatments resulted in decreased mortalities with this generation. In strain I, where both the larvae and the adults are selected, 0.02 p.p.m. parathion in the medium gave only 37% larval mortality and 0.5 mg. parathion in 100 ml. ethyl alcohol gave a total adult mortality of 47%. Even a slight increase of 0.01 p.p.m. in the medium will select the larvae drastically. The fifth larval generation was selected with 0.035 and 0.03 p.p.m. parathion in the medium. The adults were treated twice residually by the 12-liter flask method (described in the last report) before attaining sexual maturity. The dosage used here was 1.5 mg. parathion in 100 ml. ethyl alcohol, which is three times the initial dosage. The selection data are summarized in table 2.

For the second strain (II) on larval treatment only, also in the fifth generation, larval selection was made at 0.025 and 0.03 p.p.m. in the medium. This strain is being developed to determine whether drastic larval selection will contribute any protective action in the adult response to insecticide.

Topical tests on these strains, twenty-five days old flies, and on a laboratory strain for control were run to determine whether these flies were developing resistance to parathion. Based on the dosage mortality curves, there were very minor differences in the LD_{50} values of these strains. Further comparative tests were discontinued in this generation.

III. The Potential Resistance of the Oriental Fruit Fly to DDT. (C. C. Roan and S. Maeda)

When the three strains of DDT flies were received from Chemical Control, cholinesterase activities were determined. Both the P and the X strains showed a higher cholinesterase activity compared to the untreated check flies. The following generations were not treated in order to increase the fly population to a maximum.

"No-Insecticide" Strain.--This strain was maintained to serve as a control in the DDT studies. Results of the series of topical tests on this strain and the routine laboratory strain used for mass rearing are tabulated below:

LD_{50}--micrograms of DDT per gram of fly

Routine Flies	"No-Insecticide" Flies
52	47
53	51
54	56
47	34

These LD_{50} values differ so little that the "no-insecticide" strain will be discontinued and the routine laboratory strain will be used as a control for any comparative tests.

X-Strain (Larvae and Adults Selected).--The X-strain had been treated previously for three generations by the Fabri dish method before attaining sexual maturity. After omitting one selection of generation, the next generation adults were treated for the first time by the residual 12 liter flask method before attaining sexual maturity. The larvae were selected at 5 and 10 p.p.m. DDT in the carrot medium which gave 63% larval mortality. Adults were purposely selected at only the 50% level using a concentration of 200 mg. DDT in 100 ml. ethyl alcohol to insure the continuance of the next generation. With a higher population, the 6th generation was treated at 400 mg. DDT in 100 ml. ethyl alcohol, giving a 65% mortality. The population of flies has been the limiting factor in determining the level of selection.

P-Strain (Adults Selection Only).--The fifteenth generation adults totalled approximately 500 flies, when transferred to this laboratory. This and the next generation of flies were not treated in order to build up the stock culture. This strain has been previously exposed to different dilutions of DDT residues on Petri dishes, area of 78 cm^2, placed on a cylindrical test cage. This method of treatment is being continued. The seventeenth generation with a fly population of 6,000 and the eighteenth generation flies were selected after attaining sexual maturity. The results are tabulated in table 3.

The per cent mortality presented in the table is an average of twenty to forty tests. This method of testing flies results in variable mortalities. For example, at a single dosage, twenty to forty cages were set up and the per cent mortality varied from 55% to 95%. Flies surviving over a 50% mortality level were recovered for egg collection for the next generation. In the lower mortality tests, the flies were retreated.

Table 3. Dacus dorsalis exposed to various DDT residues in the selection process for DDT resistance.

DDT dilution	mg DDT/Cm^2	Average per cent mortality after 48 hrs. 17th generation	18th generation
1:1,000	26	89	96
1:2,000	14	69	72
1:3,000	9.3	43	61
1:4,000	7		40

Line Project I-o-6-3. Mass Rearing of Fruit Flies. (D. Mimoto, G. Yasuda, K. Tomei).

The total larval production for this quarter reached a maximum of 3.5 million. D. dorsalis and C. capitata have been maintained at a one-half million level each month. D. dorsalis cultures have always been satisfactory. The C. capitata cultures are quite consistent, however, there were instances of very low emergence of flies. The current production of these two species of flies seems to be adequate for supplying the needs of the various projects for experimental purposes.

The melon flies have been the most difficult of all the three species of fruit flies to culture in this laboratory. The stock cultures have continuously been supplied with wild flies collected from the fields. A change in personnel from two part-time employees to a single full-time employee in August complicated the routine of the melon stock which resulted in decreased production. The part-time employee's melon fly project became part of the daily routine and field collection depended on the available time. At the time of harvesting of host fruits, melon flies will be scarce and it will be difficult to trap wild flies. With this in mind, the staff has been varying the larval diets. With portions of the stock the larvae have been cultured only on cucumbers. For the other portion heavily

infested cucumber chunks were supplemented with different varieties of pumpkins. Pumpkins are not readily available at this time of the year. These cultures fed on different larval diets will be kept separate and comparative studies will be made.

Total Number of Larvae Produced for This Quarter

Month	D. dorsalis	C. capitata	D. cucurbitae 1/
July	465,300	572,500	334,000
August	489,100	494,000	120,000
September	478,500	495,300	148,000
Total	1,432,900	1,561,800	602,000

1/ Number of pupae; larvae are allowed to pop out and pupae are measured volumetrically.

Literature Cited

Metcalf, R. L. 1952. The Colorimetric Microestimation of Human Blood Cholinesterase and its Application to Poisoning by Organic Phosphate Insecticides. Journ. Econ. Ent. 44: (6): 883.