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TOXICITY OF INSECTICIDES TO TWO STRAINS OF *HYLEMYA PLATURA* (MEIG.) (ANTHOMYIDAE: DIPTERA)[1]

D. G. FINLAYSON AND C. J. CAMPBELL

ABSTRACT

Using the topical-application and impregnated-paper methods baseline toxicity data were obtained for male and female flies of a susceptible and a cyclodiene-insecticide resistant strain of the seed-corn maggot, **Hylemya platura** (Meig.). As shown by topical application the resistance factor with dieldrin for male and female flies was 337.8 and 342.7 respectively. However, the LC_{50} by exposure to dieldrin-impregnated papers could not be obtained for the resistant strain at the concentrations tested. There was no cross-resistance to six other insecticides: two from each of the major groups of organocarbamate, organochlorine, and organophosphorous insecticides. Both methods are useful for determining the toxicity of insecticides and offer ways for agriculturists to determine if spray practices have failed or were faulty, or if resistance is developing within a species.

INTRODUCTION

Infested onions were collected at Victoria, British Columbia in August, 1964 to establish a colony of onion maggots (*Hylemya antiqua* (Meig.)) resistant to cyclodiene insecticides. These collections yielded two species of flies: one was the onion fly; the other, somewhat smaller, was identified by the late Dr. J.G.T. Chilcott, of the Entomology Research Institute, Ottawa, as the seed-corn maggot, (*Hylemya platura* (Meig.) = *Hylemya cilicrura* (Rond.)). The onion seed had been treated with aldrin, which suggested that the smaller flies might also be resistant to the cyclodiene group of the organochlorine insecticides.

In 1961 Begg reported resistance of this type in two closely related species of root maggots, *H. cilicrura* and *H. liturata* which feed on flue-cured tobacco in southwestern Ontario. Laboratory tests at Chatham, Ontario (Harris *et al.*, 1962) with field-collected adults and comparison with laboratory-reared flies of the Chatham susceptible strain of *H. platura*, indicated that the field-collected flies were resistant to dieldrin but susceptible to diazinon. Although it was reported by Miller and McClanahan (1960) that the ratio of *H. platura* to *H. liturata* averaged 9:1 in 1958, by 1961 *H. liturata* had become the dominant species (Harris *et al.*, 1962). Attempts by Telford and Brown (1964) to compare the degree of dieldrin resistance in the two species with laboratory-reared flies proved unsuccessful. Not only were they unable to rear *H. liturata* but *H. platura* reared from collections made at Delhi proved to be as susceptible as the Chatham strain. *H. liturata* field-collected from St. Thomas and Delhi were highly resistant.

Preliminary tests (Finlayson and Noble, 1964)

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by exposing laboratory-reared flies from the Victoria source, to papers impregnated with several insecticides indicated that both males and females were resistant to dieldrin, but susceptible to diazinon and malathion. Concurrently, Harris *et al.*, (1966) obtained evidence of a low level of resistance to cylodiene insecticides in *H. platura* from the tobacco-growing areas of southwestern Ontario. More recently seed-corn maggot resistance to aldrin has been reported in Illinois (Harris, 1969).

For this experiment the susceptible strain of flies from Chatham was obtained from Dr. C. R. Harris and colonies of the Victoria and Chatham strains were reared in the laboratory to compare methods of application and the degree of toxicity of selected insecticides representing the major groups: organocarbamates, organochlorines, and organophosphorus compounds. Two methods of application were chosen; topical application to determine the median lethal dose LD_{50} for male and female flies for both strains and the impregnated-paper method, developed by the WHO for mosquitoes, to provide a simple method suitable for tests by agriculturalists. This paper reports the findings.

MATERIALS AND METHODS

Mass rearing of *H. platura* flies

Adults were maintained in cages approximately 60 x 60 x 60 cm with clear plastic on the sides and top, lumite plastic screen at the rear, and the front fitted with a small access port within a large door, (Fig. 1). The small port, with plastic screen, allowed movement of air and served for adding food and for adding or withdrawing oviposition pots.

Adult food was a 5% sugar solution in a 125 ml Erlenmeyer flask stoppered with a wick of shredded paper towelling; a mixture of molasses and condensed milk, 1:6, poured over bread in a 10 cm petri dish; and a dry mixture of Brewers' yeast, yeast hydrolysate, and soya flour, 3:1:3, spread in the bottom of a shallow 10 cm petri dish. Pollen was added to the dry mixture whenever it was available.

The breeding population was maintained at approximately 150 flies per cage and the conditions in the rearing room were maintained as close to optimum as possible: day temperature, 24°C; night temperature, 21°C; photoperiod 16 hours; and relative humidity 50-75% (Harris *et al.*, 1966).

Ovipostion pots were new one-pint (0.5 liter) ice cream containers. The pots were one-third filled with a moist peat-sand mixture (1:1), five or six 2 to 3-cm cubes of potato were added and covered with a paste of soybean flour, Brewers' yeast, and wheat flour (1:1:1), covered with the peat-sand mixture to two-thirds full, seeded with 10 to 15 dwarf pea seeds and 20 to 30 oat seeds, then covered lightly with the peat-sand mixture and kept moist. Oviposition pots were removed in four to seven days and placed in a holding cage similar to the oviposition cage, to allow development.

Flies were withdrawn from the holding cages at three to four day intervals, with a vacuum aspirator into a 1000 ml Erlenmeyer flask with a 2 cm foam pad at the bottom of the flask, held with food for 24 hours, then used for toxicity experiments. The flies tested were thus two to five days old. Surplus flies were used to determine dosage ranges and for maintinaing the colony.

Topical Application

Stock solutions were prepared by dissolving in acetone a known amount of the insecticide, of pure or technical grade. The test solutions were prepared either by serial dilution or dilution of aliquots from the stock solution. From preliminary trials to determine the approximate LD_{50}, five levels of dosages were prepared; two above, two below and the estimated median lethal dose. These doses should cause 10-90% mortality.

Impregnated Papers

Papers from two sources were used. From the WHO came papers with dieldrin or DDT dissolved in risella oil and malathion in olive oil-lonol CP. Prepared at this laboratory were papers with diazinon, in corn oil-acetone (1:2), and lindane in risella #17-trichlorethylene (1:1). Dieldrin-impregnated papers using the risella #17-trichlorethylene solvent were also prepared and tested at the laboratory. We used No. 1 Whatman filter papers, 15 cm square, which we prepared by moistening with 2 ml of the solution, the paper being held on a bed of nails. After partial drying they were attached with clips to a line in a fume hood to dry for 24 hours. The preliminary trials provided information for the range of papers needed. The papers were labelled and dated prior to treatment so that old papers would not be used.

Treatment of the flies

Two- to five-day-old flies were provided with 5% sucrose for 24 hours after removal from the emergence cage. Each replicate consisted of at least 120 flies, which were immobilized with carbon dioxide and sexed. Each replicate consisted of 10 males and 10 females for each range of the test insecticide and the same for an untreated control. With topical applications the solution was administered by two methods: by a calibrated micrometer through a #26 hypodermic needle bent at right angles and

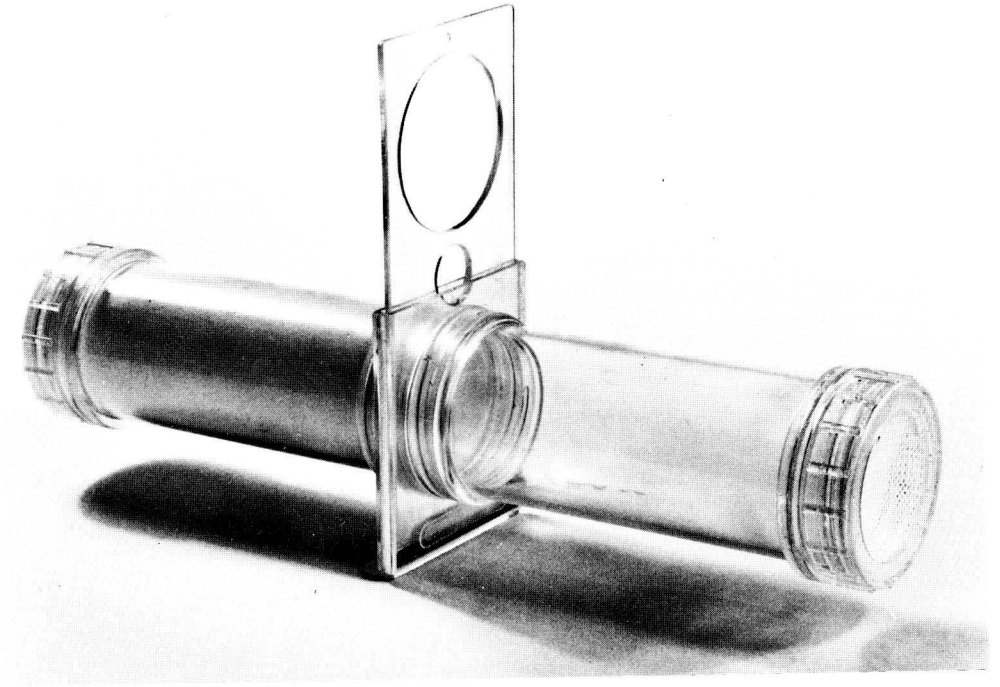

Fig. 1. Cages used for rearing large numbers of seed-corn maggot **Hylemya platura** (Meig.).

Fig. 2. WHO plastic tubes separated by slide-bar with hole exposed; Left, exposure tube with impregnated paper; Right, holding tube.

filed square at the tip, fitted to a syringe in a Syringe Microburet Model No. SB2[1]; and by a micropipette[2]. The standard dosage of 1 µl was applied to the dorsum of the thorax of the anesthetized fly and the 10 flies per dosage were placed in a plastic tube closed at each end with a screw cap fitted with plastic screening. Control flies were treated in the same manner, with 1 µl of acetone. When all the flies for a replicate had been treated they were held for one hour in the treatment area to ensure recovery of the control group from the anaesthetic. They were then transferred to the holding area for 24 hours under controlled temperature of 22 ± 2°C, relative humidity 50-60% and continuous lighting. To reduce variability the order of treatment was varied so that each group was subject to long and short periods of anaesthesia. To avoid toxic effects from carbon dioxide the flies were never held under anaesthesia for more than 30 minutes.

Impregnated papers were inserted with the treated side inward in WHO plastic tubes which were fitted with a slide bar (Fig. 2), and the 10 male or female anaesthetized flies were placed in the exposure tube. One hour later the flies were transferred to the holding tube through the hole in the slide-bar. The exposure tube was removed and the treated flies in the holding tube placed in the holding area for 24 hours.

Percentage mortality was recorded 24 hours after treatment. The criterion for death was inability to walk or fly. When mortality in the control group exceeded 20 percent the results for the complete replicate were discarded. Five replicates for each insecticide were tested, with male and female flies from both strains. Percentage mortality for each insecticide was corrected using Abbott's formula (Abbott, 1925).

Results from the topical application were averaged and the slope, LD_{50} in µg/g of fly (ppm), and the fiducial limits were calculated in accordance with Finney (1962). The resistance factor (LD_{50} Victoria strain / LD_{50} Chatham strain) was calculated for both sexes and each insecticide.

Results from the impregnated-paper method were averaged and graphs prepared by line of best fit and the LC_{50} (median lethal concentration) read from the graphs. The resistance factor (LC_{50} Victoria strain/LC_{50} Chatham strain) was calculated where possible.

[1]Micro-Metric Instrument Co., Cleveland, Ohio.

[2]Drummond Scientific Co., Bromall, Pa.

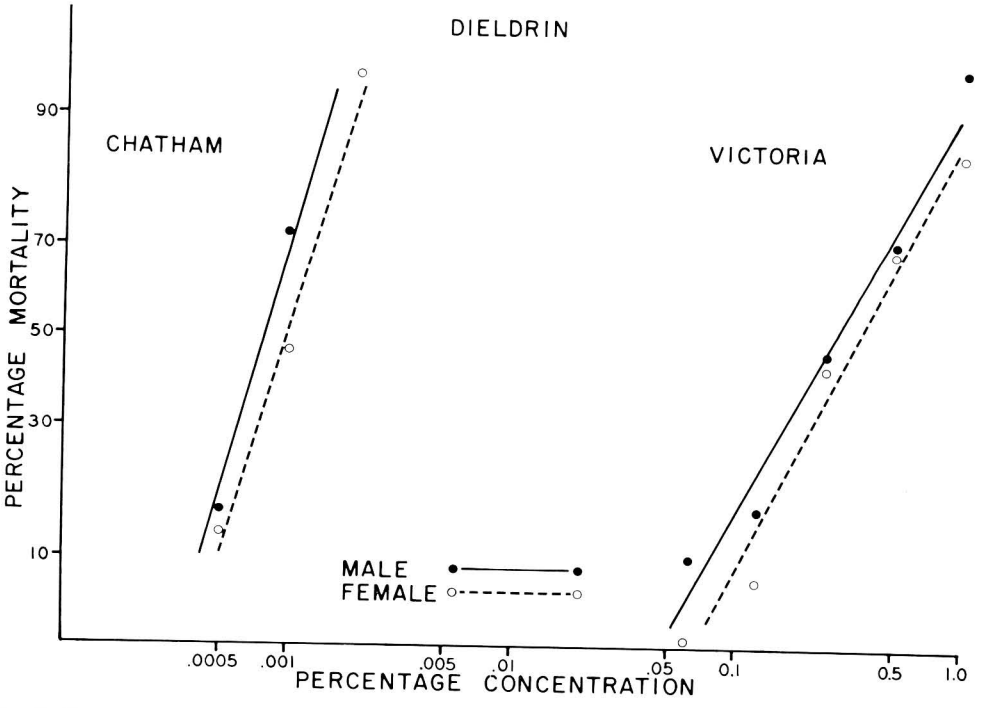

Fig. 3. Dosage-mortality regression lines, determined by topical application of dieldrin, for male and female **Hylemya platura** Chatham and Victoria strains.

TABLE 1. Toxicity of selected insecticides by topical applications to male and female flies of two strains of **Hylemya platura.**

Treatment, Strain of flies	Male				Female			
	Slope, SE of regression line	LD_{50} µg/g fly	Fiducial limits of LD_{50}	Resistance factor Vict./Chat.	Slope, SE of regression line	LD_{50} µg/g fly	Fiducial limits of LD_{50}	Resistance factor Vict./Chat.
Carbofuran								
Victoria	3.48 ± 0.61	1.36	1.00 -1.64	0.43	2.85 ± 0.40	1.58	1.16 - 1.94	0.50
Chatham	2.61 ± 0.34	3.18	2.65 - 3.81		2.69 + 0.34	3.15	2.65 - 3.76	
Carbaryl								
Victoria	2.26 ± 0.32	21.09	17.13 - 25.81	0.38	2.35 ± 0.32	25.12	20.63 - 30.76	0.28
Chatham	3.25 ± 0.39	75.58	64.52 - 88.13		2.64 ± 0.38	90.80	75.47 -110.61	
DDT								
Victoria	2.09 ± 0.24	45.94	37.69 - 56.44	3.23	2.20 ± 0.26	67.12	55.32 - 84.22	3.41
Chatham	4.38 ± 0.60	14.24	12.46 - 16.33		2.96 ± 0.56	19.70	16.30 - 26.15	
Diazinon								
Victoria	6.34 ± 0.86	0.81	0.73 - 0.90	1.05	7.48 ± 1.06	0.76	0.69 - 0.83	0.86
Chatham	3.53 ± 0.52	0.78	0.65 - 0.90		8.89 ± 0.68	0.88	0.83 - 0.95	
Dieldrin								
Victoria	2.35 ± 0.25	422.22	351.54 - 509.58	337.78	2.55 ± 0.27	500.30	421.60 - 598.96	342.67
Chatham	4.64 ± 0.57	1.25	1.06 - 1.43		4.29 ± 0.58	1.46	1.28 - 1.67	
Lindane								
Victoria	3.19 ± 0.38	20.47	17.50 - 23.92	3.44	3.42 ± 0.41	18.61	15.97 - 21.61	2.18
Chatham	2.86 ± 0.38	5.95	5.01 - 7.15		3.09 ± 0.53	8.54	7.18 - 10.71	
Malathion								
Victoria	3.75 ± 0.43	3.24	2.83 - 3.76	1.05	3.48 ± 0.41	2.62	2.26 - 3.06	0.93
Chatham	3.90 ± 0.47	3.06	2.67 - 3.54		4.90 ± 0.52	2.83	2.52 - 3.18	

Results and Discussion

Table 1 shows that male and female flies of the Victoria strain were respectively 337.8 and 342.7 times more resistant to dieldrin than the susceptible strain from Chatham. However, males and femals of the two strains were more or less equally susceptible to carbaryl, carbofuran, DDT, diazinon, lindane and malathion. The resistance factor ranged only from 0.28 for carbaryl to 3.44 for lindane. McLeod *et al.*, (1969) reported a resistance factor of 727 for aldrin in a strain of *H. platura* from Delhi near Chatham, but like ourselves, they also reported no cross-resistance to DDT and diazinon. Diazinon was the most toxic insecticide tested; the LD_{50} for both male and female flies for the two strains was less than 1.0 µg/g fly.

When we examine the dosage-mortality regression lines for topical applications (Fig. 3-6) it is quite obvious that the patterns of susceptibility for male and female flies of the same strain are similar. In all cases the lines are close and parallel or form a very shallow cross, the angle of intersection never exceeding 10 degrees. The regression lines (Fig. 3) for dieldrin inidicate that the Chatham strain is homozygous susceptible and that the Victoria strain is homozygous resistant. The slightly higher LD_{50} for lindane and DDT with the Victoria strain can hardly be interpreted as development of resistance. Nor can resistance be suspected in the Chatham strain where slightly more carbaryl and carbofuran had to be applied. These would appear to be merely strain characteristics.

The dosage-mortality curves from the impregnated paper method for 5 of the 7 insecticides are shown in Fig. 7. From this figure the LC_{50} values were read for both sexes of each strain for 4 of the 7 insecticides and the resistance factors were calculated (Table 2).

The resistance factors for the organophosphorus insecticides, diazinon and malathion, were similar by both methods. When the resistance factors were calculated for the organochlorine insecticides, DDT was 5.1 times higher by the impregnated paper method than by topical application, and lindane was 4.6 times. The resistance factor for dieldrin was hardly calculable because mortality to the Victoria strain from exposure to 4% papers was only 8.2% for males and 6.1% for females. In all probability the absorbed insecticide was detoxified.

TABLE 2. Toxicity of selected insecticides on male and female flies of two strains of **Hylemya platura** exposed to impregnated papers.

Insecticide	LC_{50} of strains of flies		Resistance factor Vict./Chat.
	Victoria	Chatham	
DDT			
male	2.10	0.114	18.42
female	2.60	0.165	15.75
Diazinon			
male	0.0275	0.0335	0.82
female	0.0390	0.0360	1.08
Dieldrin			
male	-	0.0094	-
female	-	0.0135	-
Lindane			
male	0.105	0.0096	10.94
female	0.095	0.020	4.75
Malathion			
male	1.20	1.10	1.09
female	1.20	1.94	0.62

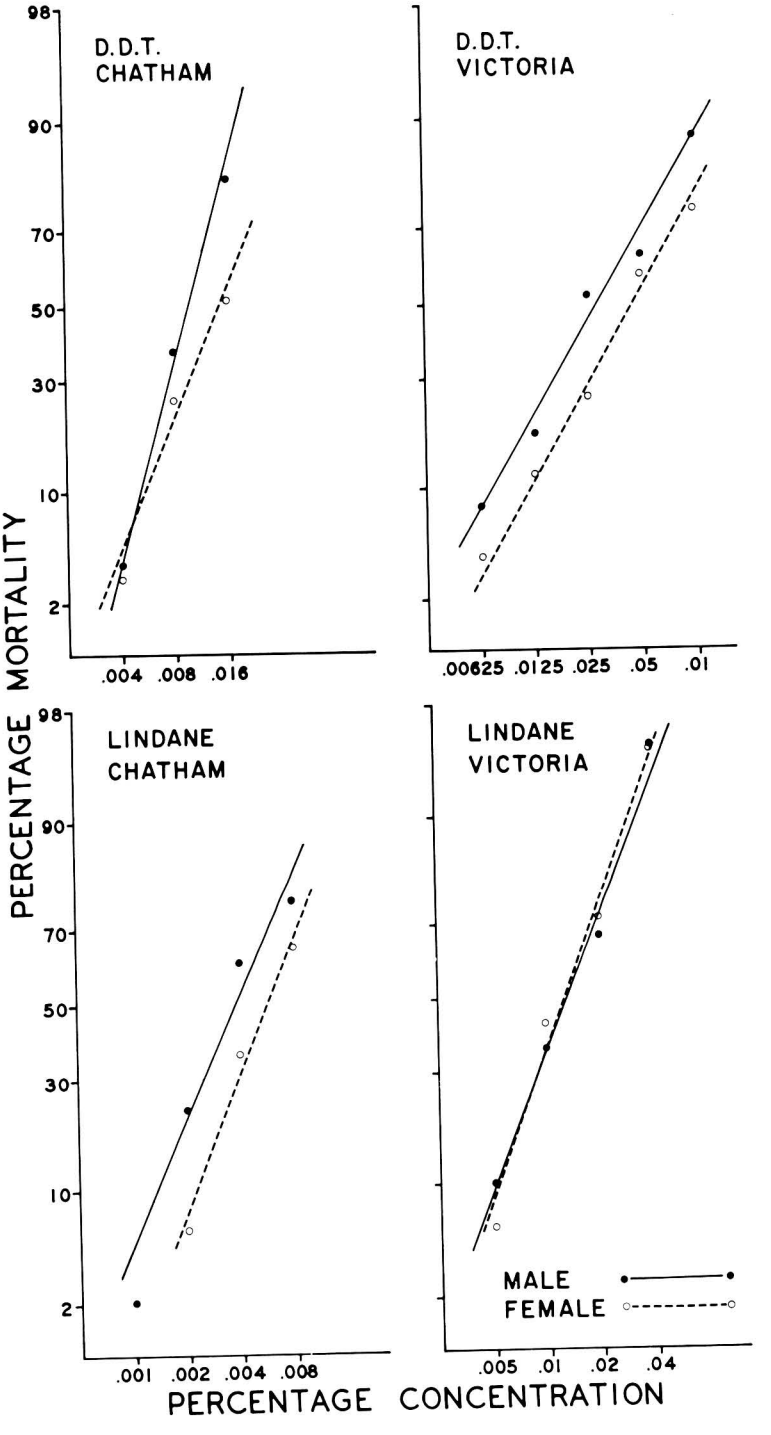

Fig. 4. Dosage-mortality regression lines, determined by topical application of organochlorine insecticides, for male and female **Hylemya platura** Chatham and Victoria strains.

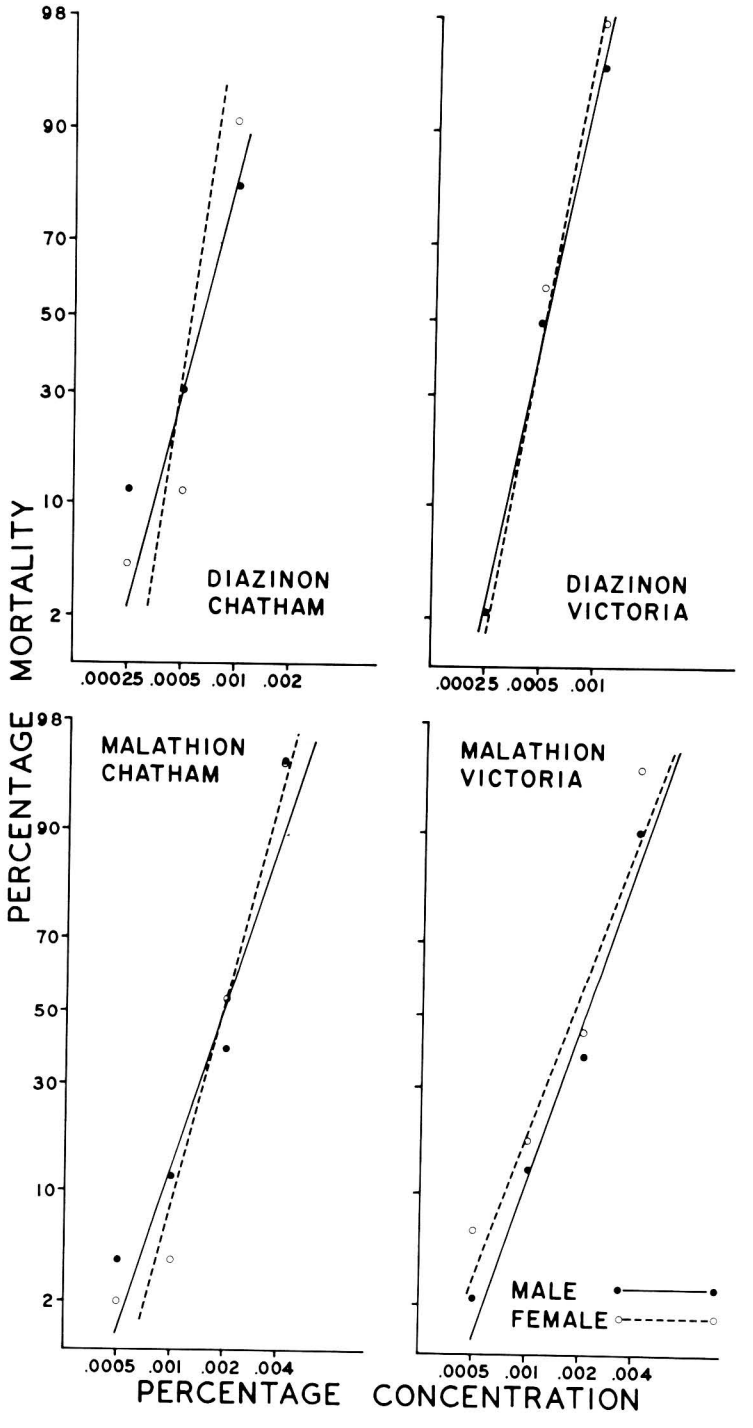

Fig. 5. Dosage-mortality regression lines, determined by topical application of organophosphorous insecticides, for male and female **Hylemya platura** Chatham and Victoria strains.

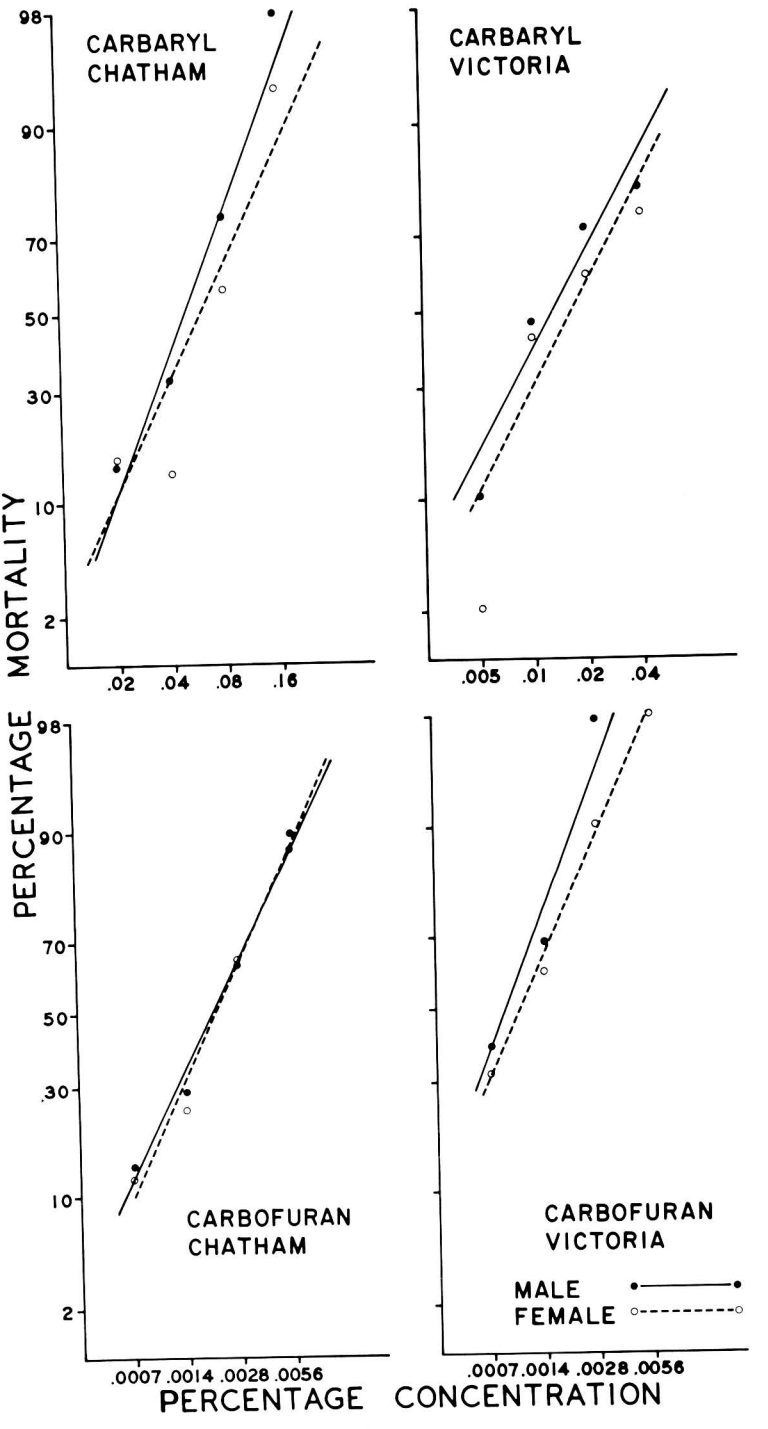

Fig. 6. Dosage-mortality regression lines, determined by topical application of organocarbamate insecticides, for male and female **Hylemya platura** Chatham and Victoria strains.

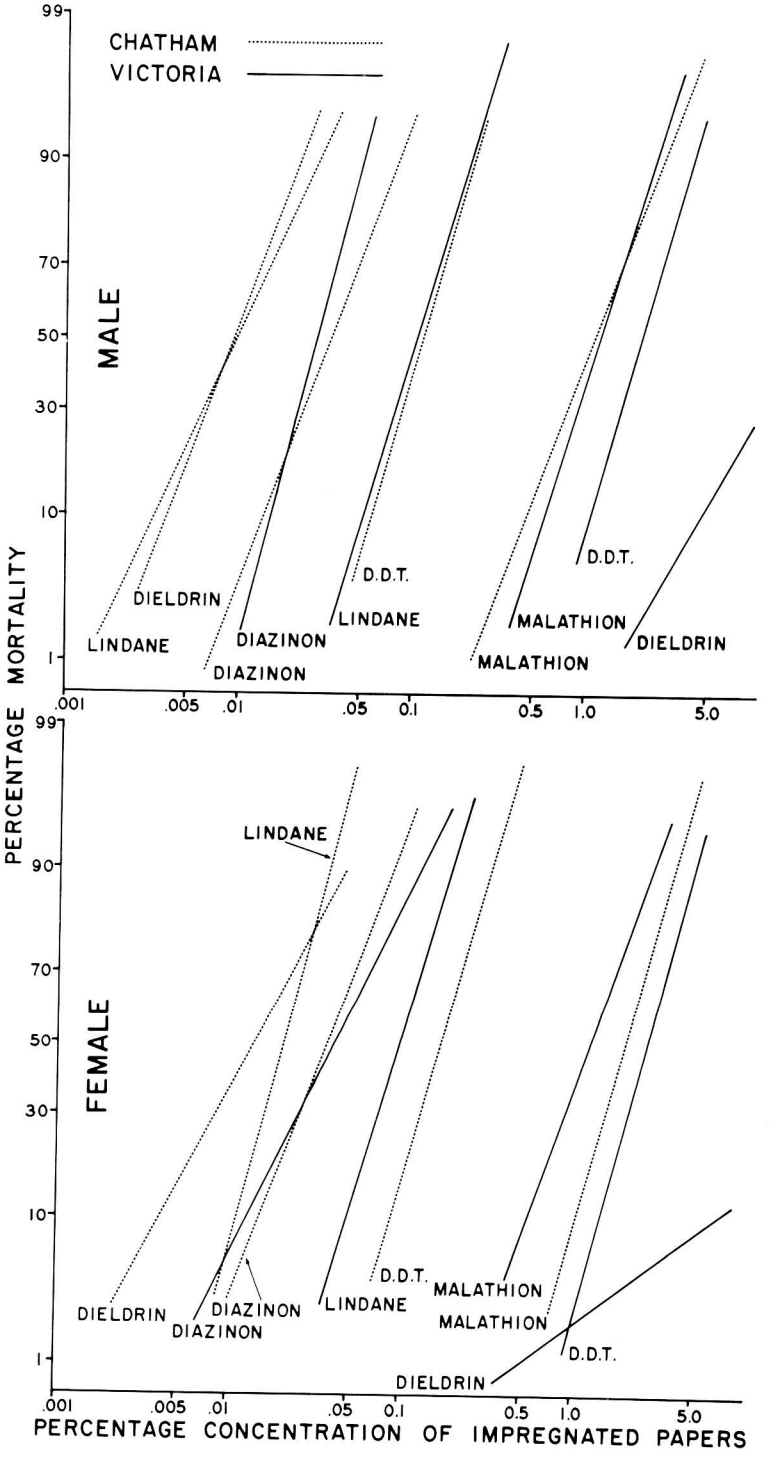

Fig. 7. Regression lines for five insecticides determined by exposure of male and female **Hylemya platura,** Chatham and Victoria strains, to impregnated papers.

We were unable to determine the LC_{50} for the organocarbamate insecticides which automatically prevented the calculation of resistance factors for the two strains. When the Victoria strain of flies was exposed to carbaryl impregnated papers, higher mortality was recorded from 1 or 2% papers than from 4% papers. When the Chatham strain was exposed to carbaryl papers, 50% mortality was not reached even with 20% impregnated papers. Exposure to carbofuran papers presented similar difficulties. Knockdown in both species occurred at various concentrations. However, by the end of the 24-hour holding period from 90-100% of the flies had recovered. The effects of topical applications were similar but to a lesser degree. At the concentrations applied topically all flies were immobilised one hour after treatment, but 24 hours later many had recovered, as shown by the dosage-mortality regression lines. Detoxification of carbofuran within the flies appears to be the only explanation.

While the impregnated-paper method affords a simple and valid technique for assessing the approximate susceptibility of strains of a species to an insecticide it is clear that the resistance factor determined from the LC_{50} could lead to wrong conclusions. The topical application of a known dosage gives more accurate results leading to firm conclusions. For indications of developing resistance the impregnated-paper method might be used, but if toxicological conclusions are to be valid then accurate dosages must be known.

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