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Differences in Acetylcholinesterase-Sensitivity to Phosphuuiidon in Mediterranean Fruit Fly Strains

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ABSTRACT

Acetylcholinesterase (AChE) from heads of the Mikve Israel strain of the Mediterranean fruit fly Ceratitis capitata was about 20% less sensitive to phosphamidon than was AChE from other field or a laboratory strain. This lower sensitivity was probably not due to an increased capacity of the homogenate to degrade phosphamidon. While resistance to organophosphorus compounds in insects is usually caused by higher rates of degradation of the compounds in resistant strains, this report describes the exceptional case of AChE-insensitivity to an organophosphorus insecticide.

In insects resistance to organophosphorus (O-P) compounds is caused by higher rates of degradation of the O-P compounds in O-P-resistant as compared to O-P- sensitive strains (O'Brien 1966). This mechanism of resistance is mediated by higher levels of hydrolytic enzymes in the O-P-resistant strains (Brown 1968).

In the housefly it could also be correlated to increased oxidation or oxidative hydrolysis mediated by cytochrome P-450 from microsomes (Nakatsugawa et al 1968, Perry 1970). On the other hand, in acarina, spider mites and cattle ticks, the mechanism of resistance to O-P compounds is a higher O-P-insensitivity of their acetylcholinesterase (AChE) (Smissaert 1964; Lee & Bathun 1966). In insects O-P resistance due to O-P-insensitive AChE is practically unknown. Only one case of a 50% less sensitive AChE from the thorax, but not from the head of O-P-resistant Blowflies Lucilia cuprina, was reported and this also was judged to be only one of the causes of their O-P resistance (Schuntner & Roulston 1968). We found that Mediterranean fruit flies collected from a locality where O-P compounds had been used extensively for many years, contained a head AChE which was less sensitive to phosnharridon than AChE obtained from heads of other strains.

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MATERIALS AND METHODS

Acetylcholine chloride was obtained from Koch-Light Laboratories; acetylthiocholine iodide from Calbiochem Chemical Company; 5, 5-dithio-bis-2-nitrobenzoic acid from Aldrich Chemical Co.; purified phosphamidon (1-chloro-1-diethylcarbamoyl-1-propen-2-yl dimethyl phosphate) a gift from Ciba Ltd., Basle, Switzerland, and malaoxon [S-(1, 2-dicarboethoxyethyl)phosphorothiolate] a gift from American Cyanamid Co., Princeton, N.J.

Guayava Psidium guayava fruits, infested with larvae of the Mediterranean fruit fly (Medfly) Ceratitis capitata Wiedeman, were collected from various localities in Israel during the autumn of 1966, 1967 and 1968. Emerging adults usually failed to oviposit or laid only very few eggs. These were reared on a dry diet of type M yeast hydrolysate mixed with sucrose (Nadel 1968). Since we did not succed in maintaining continous laboratory cultures of the field strains, these were started anew each autumn from newly infested guayava fruits. A laboratory strain obtained from the Biological Control Laboratories of the Citrus Marketing Board, Rehovot, which had adapted itself to laboratory rearing, served as control.

Determination of AChE.

Fifty heads of Medflies were ground thrice in a Potter Elvehjem glass homogeniser in 7.5 ml of NaK phosphate buffer 0.134 M pH = 7.2 for 30 seconds and cooled on ice between grindings. The homogenate was filtered through 10 layers of muslin gauze and 0.7 ml of the homogenate was taken for AChE determination by the method of Hestrin (1949). Alternatively, 2 heads of Medflies were ground as described above in 2 ml of NaKphosphate buffer 0.067 M. pH = 7.5 and filtered through 10 layers of muslin gauze. The homogenate was diluted 4 times with the same buffer solution, and 0.5 ml of the homogenate was taken for AChE determination by the method of Ellman et al. (1961), which was slightly modified (Zahavi & Tahori 1970). Alternatively eighty thoraces of

3/...

Medflies were ground as described above in Nakphosphate buffer 0.134 M, pH = 7.2. The homogenate after filtration through 10 layers of muslin gauze was centrifuged at 9000 g and 2°C for 10 minutes. The precipitate was resuspended in 7 ml of phosphate buffer.

RESULTS AND DISCUSSION

AChE from Medfly heads and thoraces, similar to AChE from Diptera in general, was very sensitive to phosphamidon (Table I). Later on experiments showed that phosphamidon was not the most potent O-P inhibitor of Medfly AChE. Malaoxon which showed the same inhibition pattern as phosphamidon for AChE's from Medflies, aphids and spider mites, was about 30 times more potent to Medfly AChE than phosphamidon.

AChE from heads of Medflies from the Mikve Israel strain was about 20% less sensitive to phosphamidon than was AChE from heads of other field or a laboratory strain (Table II). As no decrease in the extent of inhibition occurred when the reaction period was prolonged from 10 to 20 or even 30 minutes (Table II), it seems unlikely that the lower sensitivity of AChE of the Mikve Israel strain was due to an increased capacity of the homogenate from this strain to degrade phosphamidon.

4/...

Medflies from an area where O-P compounds have been used extensively for many years possessed an AChE more insensitive to O-P compounds than that of other strains. While field resistance to O-P compounds could easily develop from such an insensitivity, to the best of our knowledge no O-P resistance in Medflies has yet been reported.

With the exception of the one report by Schuntner & Roulston (1968), O-P-insensitivity of their AChE is an unknown mechanism in O-P resistance in insects. A correlation of our in vitro data with in vivo toxicity tests by topical application could not be carried out, since we were unable to rear the Mikve Israel strain in captivity and thus to obtain the fly in numbers large enough for conclusive toxicity tests. For this reason, we were also unable to confirm the phosphamidon insensitivity data with malaoxon which is a far better inhibitor of Medfly head AChE than is phosphamidon. However, since this report describes only the second case of AChE insensitivity to an O-P compound in an insect, we deemed it of interest to report these preliminary results in full.

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Table I. Inhibition of AChE of Medflies

	ID_{50} and ID_{80} in 10^{-7} M		
	head homogenate	thorax particles	thorax soluble
ID_{50}	2.0	2.2	2.8
ID_{80}	4.8	5.76	8.64

Data are averages of 4 to 7 experiments. ID_{50}s and ID_{80}s were obtained by plotting % inhibition against 3 to 4 concentrations on semilogarithmic paper. AChE activity measured in μmole/mg protein/hour was for: Medfly head homogenate 28.5; Medfly thorax particles 1.8; Medfly thorax soluble 1.5; AChE was determined by the method of Hestrin (1949).

Table II Inhibition of head AChE from various Medfly strains by phosphamidon

Concentration of phosphamidon in M	% inhibition																	
	Laboratory strain			Zikim			Beit Dagan			Ramat Hasharon			Mikve Israel					
	A	B	C	A	B	C	A	B	C	A	B	C	A 1	A 2	B 1	B 2	C 1	C 2
10^{-7}	30	24.5	30	27	29	30	27.5	27.5	27.5	27.5	27	28.2	29.5	22.5	26	25	28.7	25.5
$1.5x10^{-7}$	42	41.5	42.5	37.5	40.5	42	35.5	38	36.5	37.2	38.5	39.8	35.5	32	35.5	36.5	36	38
$1x10^{-7}$	42	43.5	46.5	43	40.5	43	47.5	48.5	47	45.7	45.3	46.5	40	37.5	38.7	37.5	40.7	42
$3x10^{-7}$	61	61.6	63.5	57	55.5	58	62	64	63	61.7	61.8	61.7	57	53.5	55.2	52.5	57.7	55
$4x10^{-7}$	71	72	64	69	66	68.5	71	73	73	69.5	69.3	69.8	64.7	64.2	62.4	65.4	65.4	69
ID_{50} in 10^{-7}	2.05	2.2	2.15	2.3	2.4	2.2	2.15	2.1	2.15	2.15	2.15	2.1	2.5	2.65	2.5	2.75	2.5	2.55

A time of reaction 10 minutes; B time of reaction 20 minutes; C time of reaction 30 minutes

AChE was determined by the method of Ellman et al (1961).

The difference in % inhibition between the Mikve Israel and all the other strains was found to be significant at the 99 % level of significance for each concentration of phosphamidon.