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RESISTANCE TO TEMEPHOS, AN ORGANOPHOSPHOROUS INSECTICIDE, IN *CULEX PIPIENS* FROM TUNISIA, NORTH AFRICA

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ABSTRACT. Resistance to temephos, an organophosphorous insecticide (OP), was found to be low (2-fold) in 2 *Culex pipiens* populations collected in Sayada (mid-eastern Tunisia). This resistance was synergized by an esterase inhibitor (DEF). Two sets of over-produced esterases (A2-B2 and A4-B4), known to be involved in resistance, were identified in almost 50% of the examined insects. In addition, 3% of insects had an insensitive acetylcholinesterase. After selecting larvae of one of the samples (ES) with temephos for 6 generations, a 9-fold increase in resistance was observed, and all mosquitoes were found to carry esterases A2-B2 and an insensitive acetylcholinesterase. These results must be considered in future mosquito control programs, since 2 of the identified genes can lead to high resistance to several organophosphorous insecticides.

Culex pipiens Linn. is widely distributed in Tunisia, but no data are available on its resistance status. In this report, we have analyzed the resistance to temephos, an organophosphorous insecticide (OP), of 2 populations (ES and PS) collected as larvae in Sayada (mid-eastern Tunisia) in October 1990.

Bioassays were performed on the collected (F0) 4th-instar larvae, using alcoholic solutions of temephos (American Cyanamid, Princeton, NJ) as described by Raymond et al. (1986). Mortality data were analyzed by using the log-probit program of Raymond (1993), based on Finney (1971). This program provides LDs and slope for each mortality line, it tests parallelism between 2 or more mortality lines and it computes resistance (or synergism) ratios with their 95% confidence limits (CL). Temephos mortality curves of ES and PS larvae (Table 1) were linear ($P > 0.05$) as was the mortality line of susceptible larvae from the reference S-LAB strain (Georghiou et al. 1966). Parallelism between ES and PS, ES and S-LAB, and PS and S-LAB mortality lines was not rejected at the 5% level. PS larvae were slightly and significantly more resistant than ES larvae (1.3-fold, CL = 1.2–1.4), and both ES and PS larvae were also more resistant than S-LAB: 1.6 (CL = 1.1–2.8) and 1.9 (CL = 1.4–3.3), respectively.

The physiological mechanisms responsible for the observed resistance were investigated in testing the synergism of S,S,S-tributylphosphorotritrioate (DEF, Interchim, Montluçon, France) on temephos resistance and in researching eventual over-produced esterases known to be involved in resistance to OPs. Exposing larvae to a standard dose (0.1 mg/liter) of DEF 4 h before the addition of temephos decreased the tolerance in ES, PS and S-LAB samples. For the 3 samples, parallelism of mortality lines obtained in the presence of temephos and DEF and of temephos alone was not rejected at the 5% level; the synergism ratios were 7.3 (CL = 5.0–11.4) for ES, 9.4 (CL = 6.5–15.0) for PS and 5.7 (CL = 3.8–9.4) for S-LAB. Since ES and PS synergism ratios are higher than that of S-LAB, it can be concluded that in these 2 strains, the decrease in temephos tolerance due to DEF is more important than in S-LAB, suggesting the presence of a more efficient detoxication mechanism by either esterases or glutathione transferases.

Over-produced esterases were investigated in single adult homogenates using starch gel electrophoresis (Pasteur et al. 1988). Two sets of associated over-produced esterases with the same electrophoretic mobility as A4 and B4 in VIM strain (Poirié et al. 1992) and as esterases A2 and B2 in SELAX strain (Wirth et al. 1990) were identified (Table 2): esterases A4-B4 were found in 38 and 33% of the mosquitoes of the ES and PS samples, respectively; esterases A2-B2 in 14 and 16%, respectively. Over-production of esterases is due to amplification of a large DNA fragment (or haplotype) containing the structural esterase B gene (Mouchès et al. 1990). Poirié et al. (1992) have shown that in VIM and CYPRUS strains, mosquitoes which have electrophoretically identical over-produced esterases possess different amplified haplotypes, hence their different names. The esterases named A4-B4 in the present study can therefore be "true" A4-B4 or A5-B5 or a mixture of the 2. The low temephos resistance observed in the 2 Tunisian samples suggests, however, that if esterases A5-B5 are present, their frequency is low since these esterases are known to confer an 82-fold resistance to this insecticide whereas A4-B4 confers only a 1.6-fold resistance (Poirié et al. 1992).

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Table 1. Resistance characteristics of Sayada (Tunisia) populations of *Culex pipiens*.

Sample	LD_{50} (mg/liter)		Slope	H[1]	RR[2]
Temephos					
S-LAB	0.0018	(0.0017–0.0019)[4]	5.56 ± 0.39	1	1.0
ES-SAYADA(F0)	0.0027	(0.0025–0.0029)	3.87 ± 0.28	1	1.5[5]
PS-SAYADA(F0)	0.0034	(0.0032–0.0036)	3.98 ± 0.25	1	1.9[5]
ES-SAYADA(F6)[3]	0.023	(0.021–0.026)	3.87 ± 0.24	1	13.4[5]
Temephos + DEF					
S-LAB	0.00032	(0.00028–0.00036)	0.70 ± 2.63	1	1.0
ES-SAYADA(F0)	0.00036	(0.00033–0.00039)	3.98 ± 0.23	1	1.1
PS-SAYADA(F0)	0.00035	(0.00032–0.00038)	3.40 ± 0.23	1	1.1

[1] Heterogeneity factor.
[2] Resistance ratio.
[3] Selected with temephos each generation.
[4] 95% confidence limits.
[5] Significantly different from 1 at $P = 0.05$.

Finally, the microtiter plate test of Raymond et al. (1985) disclosed, among the 29 individuals examined in each Tunisian sample, the presence of one mosquito heterozygous for the presence of insensitive acetylcholinesterase (Ace^{RS}).

Although the 2 Sayada populations displayed a low (2-fold) resistance to temephos, our study revealed the presence of at least 3 different resistance genes: insensitive acetylcholinesterase (Ace^{R}) and the associated esterases A4-B4 and A2-B2. These genes have all been observed in other countries (Raymond et al. 1991a, 1991b; Poirié et al. 1992) and thoroughly studied on homozygous laboratory strains. In particular, it was shown that Ace^{R} and esterases A2-B2 provide 8.8- and 62-fold resistance to temephos, and 97- and 32-fold resistance to chlorpyrifos, respectively (Raymond et al. 1986, Wirth et al. 1990). These genes, therefore, may rapidly impede mosquito control programs based on OP treatments since their frequency is likely to increase drastically in a few generations. This was shown in selecting the ES population with temephos doses killing 60–80% of the larvae. After 6 generations of selection, temephos LD_{50} rose to 0.023 mg/liter, which corresponds to a 9-fold increase in resistance (Table 1). All mosquitoes of the selected ES strain were found to possess esterases A2-B2 as well as an insensitive acetylcholinesterase (Table 2).

In conclusion, our study indicates that it would be important for the development of efficient mosquito control programs in Tunisia to determine precisely the geographic distribution of each resistance gene.

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Table 2. Resistance genes detected in the Tunisian populations of *Culex pipiens* from Sayada.

Resistance genes	PS (F0)	ES (F0)	ES (F6)
Over-produced esterases			
None	30 (0.52)	31 (0.53)	0 (0)
A4-B4	19 (0.33)	19 (0.33)	0 (0)
A2-B2	9 (0.16)	5 (0.09)	28 (1.0)
A4-B4 and A2-B2	0 (0)	3 (0.05)	0 (0)
Total	58	58	28
Insensitive acetylcholinesterase			
Ace^{SS}	28 (0.97)	28 (0.97)	0 (0)
Ace^{SR}	1 (0.03)	1 (0.03)	35 (0.60)
Ace^{RR}	0 (0)	0 (0)	23 (0.40)
Total	29	29	58

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