MECHANISMS OF INSECTICIDE RESISTANCE IN FIELD POPULATIONS OF CULEX PIPIENS FROM ITALY

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ABSTRACT. Results of a study on organophosphate (OP) resistance carried out on 4 Italian field populations of *Culex pipiens* are reported. The A1, A4-B4 and/or A5-B5 nonspecific esterases and insensitive acetylcholinesterase (AChE) were detected in our samples. A2-B2 esterases previously recorded in Italy were not observed. The A4-B4 and/or A5-B5 esterases were first found in Italy where they are at present widespread. Both nonspecific esterases and insensitive AChE are involved in OP resistance, although the high level of OP resistance observed in the Padova population could be correlated with both a high frequency of insensitive AChE and A5-B5 esterases.

INTRODUCTION

Overproduction of detoxifying nonspecific esterases is a widespread mechanism of organophosphate resistance in Culex pipiens Linn. mosquitoes (Raymond et al. 1992), and selection for insensitive acetylcholinesterase (AChE) has also been recorded in Europe (ffrench-Constant and Bonning 1989, Raymond et al. 1987). In Culex pipiens, the nonspecific esterases are encoded by 2 closely linked loci, named A and B according to substrate preference of the enzymes (α - or β -naphthylacetate). Overproduction of the B esterases is due to gene amplification (resulting in the B1, B2, B4 and B5 alleles, which generally are in linkage disequilibrium with specific A alleles) and is often responsible for organophosphate resistance in Culex mosquitoes (Mouchés et al. 1986, Raymond et al. 1991, Poirié et al. 1992). In particular, the amplified esterase B2 allele (which is in linkage disequilibrium with the esterase A2 allele) has originated from a single mutational event and spread thereafter. The A2-B2 esterases, present in Asia, Africa and North America, have also been recorded in Italy and France (Raymond et al. 1991).

In Italy, organophosphate resistance of *Culex pipiens* has been studied in only 3 areas: Latina, Latium (Villani et al. 1982); Lucca, Tuscany (Bonning et al. 1991); and in Sardinian urban areas (Marchi and Addis 1990). We have undertaken a comprehensive geographical survey of *Culex pipiens* mosquitoes in order to: 1) reevaluate the molecular mechanisms of organophosphate resistance that have previously been reported in *Culex pipiens* populations from Latina and Lucca and, 2) identify the current ones that have been selected in the populations from areas never studied before. Padova (Venetia) and Bovalino Marina (Calabria) have been chosen as reflecting opposite situations in the mosquito control activities. Our survey of the distribution of overproduced nonspecific esterases as well as of insensitive acetylcholinesterase is presented here.

MATERIALS AND METHODS

The location of the study areas are shown in Fig. 1. Batches of 3rd-4th instar *Culex pipiens* larvae were collected by dipping in urban areas from polluted water sources such as sewage or drainage channels and reared in our insectary until reaching adulthood. A long-established laboratory strain, ISS was employed as a standard susceptible strain for comparison.

Resistance to chlorpyrifos and temephos organophosphate insecticides was determined on late 3rd-early 4th instar larvae (sample size = 480 larvae/population) using WHO bioassay

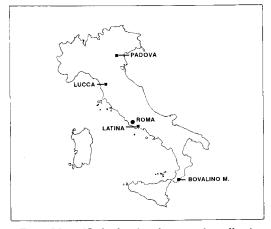


Fig. 1. Map of Italy showing the mosquito collection localities.

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test procedures (World Health Organization 1981). The LC_{50} values were calculated by a computer-assisted program. Esterases were studied on adults emerged from the collected larvae. They were homogenized and submitted, along with reference strains (A1 Barriol, A2-B2 SeLax and A5-B5 Cyprus), to horizontal starch gel electrophoresis in Tris-maleate-EDTA (Pasteur et al. 1981). Gels were incubated in phosphate buffer (pH 6.5) containing 0.05% (final concentration) of α -naphthylacetate and β -naphthylacetate prior to the addition of 0.3% (final concentration) Fast Garnett GBC. The acetylcholinesterase assay was carried out according to the method described by Raymond et al. (1985) using propoxur as an inhibitor (6.6 10^{-2} **M**).

RESULTS

The susceptibility values of the various samples of natural populations of Culex pipiens to temephos and chlorpyrifos are presented and compared in Table 1. In comparison with data obtained in 1986 (Villani et al. 1986), the Lucca population was about 15-fold less resistant to chlorpyrifos, and about 4-fold more resistant to temephos (resistance ratio, RR = 9.4 and 3.5, respectively). In the Latina population, the levels of resistance present to both chlorpyrifos and temephos observed in 1986 were the same (RR = 4.5 and 1.7, respectively). The Padova and Bovalino Marina populations were never studied before. When compared with the ISS (susceptible) strain, the Padova population is resistant to both chlorpyrifos and temephos (RR = 122.7and 83.3, respectively), while the Bovalino Marina population demonstrated a susceptibility higher than that showed by the reference strain (RR = 0.6 and 0.7, respectively) to both the organophosphate compounds.

The frequencies of mosquitoes displaying nonspecific esterases and of insensitive AChE in the examined field populations are shown in Table 2. The most common electrophoretic pattern so far observed and never described in Italy corresponds to that shown by the Cyprus reference strain (which possess the A5-B5 esterases), but could represent either A4-B4 or A5-B5 esterases due to their identical electrophoretic mobility (Poirié et al. 1992) (Fig. 2). However, these esterases differ in the resistance levels they confer to chlorpyrifos or temephos (Table 3). As A5-B5 esterases provide a relatively high temephos resistance, they are probably absent in Lucca, Latina and Bovalino Marina populations. The detected esterases are probably A4 and B4 (in addition of A1) in these 3 samples.

 Table 1. Comparative susceptibilities and resistance

 ratios (RR) of Culex pipiens populations to

 chlorpyrifos and temephos.

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Insecticide Population	LC ₅₀ * (95% CL)	Slope	χ²	DF	RR	
Chlorpyrifos						
Padova	80.9	1.61	2.56	2	122.7	
	(69.3-94.3)					
Lucca	6.2	2.15	4.13	2	9.4	
	(5.3 - 7.4)					
Latina	3.0	2.65	1.16	2	4.5	
	(2.3 - 3.8)					
Bovalino M.	0.39	5.59	2.71	2	0.6	
	(0.13 - 0.90)					
ISS	0.66	2.81	0.35	1	—	
	(0.41 - 1.1)					
Temephos						
Padova	20.1	1.92	1.00	1	83.3	
	(15.7 - 25.8)					
Lucca	0.85	2.86	0.04	1	3.5	
	(0.30 - 2.3)					
Latina	0.40	4.10	0.53	1	1.7	
	(0.20 - 0.79)					
Bovalino M.	0.17	2.81	0.07	1	0.7	
	(0.14-0.21)					
ISS	0.24	1.73	7.32	1		
	(0.21 - 0.28)					
	0					

* As mg/liter $\times 10^{-3}$.

Table 2. Frequencies of mosquitoes displaying highly active esterases and of insensitive acetylcholinesterase (AChE) in the populations

	analyze	ed"	
Field popu- lation	A1 esterase	A4-B4 or A5- B5 es- terases	Insensitive AChE
Padova	0.3 (86)	0.65 (86)	0.56 (32)
Lucca	0.21 (58)	0.57 (58)	0.28 (32)
Latina	0.16 (31)	0.55 (31)	0.12 (32)
Bovalino M.	0.09 (22)	0.27(22)	0.0 (19)

* Values in parentheses are numbers tested.

In the Padova sample, the high temephos resistance is best explained by the presence of A5-B5, unless another undetected temephos resistance gene exist.

DISCUSSION

The data obtained in this survey show differences in organophosphate resistances, in esterase electrophoretic patterns, as well as in the altered AChE levels, due to the variations in vector control activities carried out in the areas so far examined. Efforts to control *Culex pipiens* mosquitoes in Italy vary greatly, depending on local vector agencies and resources (chlorpyrifos

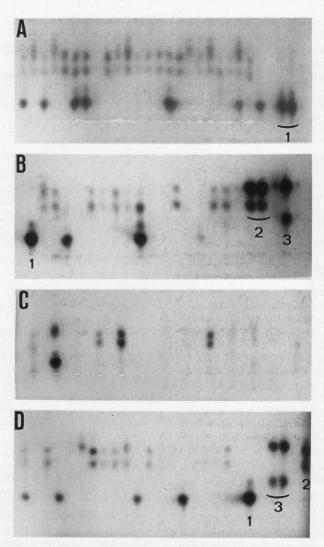


Fig. 2. Nonspecific esterases in 4 *Culex pipiens* populations from different regions of Italy. A = Padova; B = Latina; C = Bovalino M; D = Lucca. Reference strains: 1 = Barriol (A1 est.); 2 = Cyprus (A5B5 est.); 3 = SeLax (A2B2 est.).

 Table 3. Resistance ratios for chlorpyrifos and temephos in Culex pipiens reference strains determined by comparison with the S-Lab* susceptible strain LC₅₀.

Strain	Chlorpyrifos	Temephos	Reference
MSE (AChE)	97	8.2	Raymond et al. (1985)
S54 (A1)	3.2	1.6	Raymond et al. (1985)
VIM (A4-B4)	6.6	1.7	Poirié et al. (1992)
Cyprus (A5-B5)	95	82	Poirié et al. (1992)
SeLax (A2-B2)	25	22	Wirth et al. (1990)

* Georghiou et al. (1966).

and temephos are the most used insecticides). For instance, since 1986, treatments with chlorpyrifos were discontinued in Lucca (thus accounting for the reduced level of resistance to that insecticide) and replaced by the use of temephos during the following 3 years. In 1989, all types of treatments were stopped. In contrast with previously reported data (Villani and Hemingway 1987, Bonning et al. 1991), the A2-B2 esterases were not present in our sample of mosquitoes collected in Lucca (as well as in other Italian areas), although their presence at very low frequencies cannot completely be ruled out. Apparently these recent resistance genes have started to spread in the Lucca area due to organophosphate usage, and have now decreased to an undetected level following the end of the insecticide control. This is in contrast to the French situation where the same A2-B2 esterases are still extending their geographical range, particularly in temphos treated areas (Rivet et al. 1993). The frequency of the A1 allele still accounted for 0.21 vs. the 0.36 frequency reported in 1986 by Villani and Hemingway (1987).

Since 1985, control activity was reduced in Latina. However, it is probably still adequate for maintaining the same degree of organophosphate resistance as that observed in 1986; nevertheless, the frequency of the A1 esterase accounted for 0.16 vs. the 0.6 frequency observed in 1986. On the contrary, in Padova, where vector control activity is regularly performed using chlorpyrifos since 1989, a high level of organophosphate resistance was observed. In the Padova population 3 mechanisms are operative: the frequencies of the A1 esterase, the altered AChE, and the A4-B4 or A5-B5 esterases, accounting for 0.3, 0.56 and 0.65, respectively. In the field population from Bovalino Marina, where larval control activities are neglected, only a relatively low frequency of A1 esterase (0.09) and no altered AChE were detected, while the A4-B4 or A5-B5 esterases showed a 0.27 frequency.

All the resistance genes have decreased in frequency in areas where the control units have reduced their use of organophosphate insecticides. This is probably associated with resistance genes, which are at a disadvantage compared with susceptible genes when no insecticide is present. This indicates that all these resistance mechanisms were involved in organophosphate resistance, as expected from the theoretical work on interaction of various resistance genes by Raymond et al. (1989).

The A4-B4 and A5-B5 esterases have only been detected in western (for A4-B4) and east-

ern (for A5-B5) Mediterranean countries, where they probably originated (Poirié et al. 1992). It is possible that they are both present in Italy, because A5-B5 provides a relatively high resistance to organophosphates (and particularly to temephos). These esterases are probably absent from Lucca, Latina and Bovalino Marina samples (so that the esterases detected are A4-B4), and probably present in the Padova population. Although other possibilities potentially exist to explain a high temephos resistance in Padova (like another allele of insensitive AChE), it is not surprising to find a mixture of A4-B4 and A5-B5 in a country occupying a central position within the Mediterranean area.

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