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SUSCEPTIBILITY OF ITALIAN POPULATIONS OF *AEDES ALBOPICTUS* TO TEMEPHOS AND TO OTHER INSECTICIDES

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ABSTRACT. We evaluated larval and adult susceptibility to 5 insecticides commonly used for control of *Aedes albopictus,* by using 15 field-collected populations from northern and central Italy, and we compared these data, collected in 2002, with those from evaluations conducted in 1992–93, 1996, and 1998–99. Larvae were tested for susceptibility to temephos and to the conventional diagnostic dosages of chlorpyrifos and fenthion proposed by the World Health Organization for *Aedes aegypti.* Adults were exposed to the diagnostic dosages of deltamethrin and permethrin. Overall, all of the populations tested in 2002 were still susceptible to temephos: median lethal concentration (LC_{50}) values ranged from 0.0026 to 0.0085 mg/liter, and LC_{99} values ranged from 0.0093 to 0.023 mg/liter. These populations were also fully susceptible to chlorpyrifos and fenthion, and adults were susceptible to deltamethrin and permethrin. When comparing these data with those from previous evaluations, we observed a slight yet progressive increase in the LC_{50} and LC_{99} values of susceptibility to temephos, and these values are approaching the diagnostic dosage of 0.02 mg/liter proposed for *Ae. aegypti.* However, the implications of these findings need to be considered in light of the results of previous studies that have shown that larval *Ae. albopictus* are less susceptible than *Ae. aegypti* to organophosphate insecticides.

KEY WORDS *Aedes albopictus,* insecticide susceptibility, larvicides, temephos, Italy

INTRODUCTION

Aedes albopictus (Skuse) was 1st reported in Italy in 1990 (Sabatini et al. 1990). The main site of entry into the country was the area of Padova (a city in northern Italy), where the country's major tire-retreading companies are located (Dalla Pozza et al. 1994, Romi et al. 1999). From there, *Ae. albopictus* gradually spread throughout nearly all of the northern and central regions, facilitated by the domestic trade of used tires (Romi et. al. 1999). Only a few restricted foci of infestation have been reported in the southern regions of the country, where the climatic conditions are not favorable for the mass breeding of this species (Romi 2001).

The establishment of *Ae. albopictus* in Italy has caused great concern among public-health authorities (Knudsen et al. 1995, 1996). In 1991, a reference center for the surveillance of this species was created at the Department of Parasitology of the Istituto Superiore di Sanità (ISS). Monitoring and control currently are carried out by local public-health agencies (Aziende Sanitarie Locali [ASL]) or by town governments, by following the guidelines provided by the Reference Center (Romi 1996). Control activities consist of reducing the sources of these mosquitoes and performing larvicidal treatment and focal adulticide spraying. Larvicidal treatment focuses on public sewer-drain basins, and it consists mainly of treatment with temephos every 2–3 wk, from May to October (other organophosphate larvicides are seldom used). Adulticide spraying was mainly conducted in August–September (i.e., the period of peak adult densities of *Ae. albopictus*) or in cases of heavy infestation, and the most commonly used insecticides are deltamethrin and permethrin.

In 1992–93, when intensive larvicide treatment began, the Reference Center of the ISS established the baseline of susceptibility to temephos for the major populations of *Ae. albopictus* at that time (i.e., Genova, Padova, and Brescia). To monitor potential changes in susceptibility to temephos, in 1996 and in 1998–99, bioassays on larval susceptibility of *Ae. albopictus* were conducted.

The objective of the present study was to evaluate current larval and adult susceptibility to the 5 most commonly used insecticides (i.e., temephos, chlorpyrifos, fenthion, deltamethrin, and permethrin) of 15 field-collected populations of *Ae. albopictus* from northern and central Italy. The data obtained were compared with the available data from previous evaluations and are reported and discussed in this paper.

MATERIALS AND METHODS

Mosquito populations: Fifteen field populations of *Ae. albopictus,* collected in 8 heavily infested areas of Italy, were selected for this study; these populations included those that had been studied in 1992–93, 1996, and 1998–99. The geographical locations of the study areas, identified by the name of the province, are shown in Fig. 1.

Most of the populations came from northern regions of the country, in particular: the cities of Genova (n = 2 sites), Brescia (n = 2), and Padova (n = 2), and the provinces of Udine (n = 1), Bologna (n = 4), and Forlì (n = 1); central Italy is represented by 1 site in the province of Grosseto and 2 sites in the city of Rome (those areas, in which samples were collected from more than 1 site, were the most heavily infested). Eggs were collected from ovitraps used by the ASLs and town governments for routine monitoring. To allow egg hatching, the Masonite® strips, used as a surface for egg

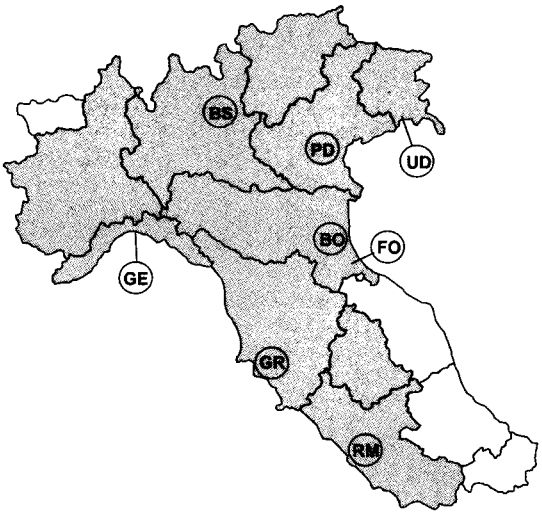

Fig. 1. Map of north and central Italy showing location of the areas selected (circles) for testing the susceptibility of 15 populations of *Aedes albopictus* to temephos and to other insecticides. The regions where foci of the species have been reported are indicated in gray. Provinces are abbreviated as in Table 1.

laying, were taken from the ovitraps, brought to the Reference Center, and placed in plastic trays (30 × 15 × 10 cm) containing 1 liter of dechlorinated tap water. The larvae were reared in a climatic chamber at a temperature of 27 ± 1°C and a photoperiod of 16:8 h light : dark until reaching adulthood.

Insecticide susceptibility tests: In all 4 periods (i.e., 1992–93, 1996, 1998–99, and 2002), the bioassays were performed between June and September. Testing for larval susceptibility to temephos was carried out by following the standard method of the World Health Organization (WHO 1981a). Specifically, testing was conducted in a climatic chamber with the above-reported temperature and photoperiod. Tests consisted of at least 3 replicates per concentration and 5 or 6 different concentrations of temephos (ranging from 0.001 to 0.02 mg/liter) per population, plus untreated controls. Batches of 20 larvae were exposed to different dosages of temephos in glass beakers each containing 250 ml of dechlorinated water. After 24 h, larval mortality was checked. In 2002, about 6,000 late 3rd- to early 4th-stage larvae were tested (a mean of 360 larvae per population). By using a software created at the Reference Center, a log-probit regression line for temephos was obtained and median and 99% lethal concentrations (LC_{50} and LC_{99}, respectively) were established. Regression lines were determined from a minimum of 3 to a maximum of 5 points giving a rate of mortality comprised between 1% and 99%. All of the tests reported in this study provided regression lines with values of $\chi^2 < 5$, degree of freedom ≥ 1, and slope < 2. For the evaluation of susceptibility to chlorpyrifos and fenthion (only occasionally used as larvicides) the larvae were exposed to the diagnostic dosages proposed by WHO (1981a) as conventional concentrations for discriminating between susceptibility and resistance for *Aedes aegypti* (L.) (0.01 and 0.05 mg/liter, respectively) and to half of the same diagnostic dosages (i.e., 0.005 and 0.025 mg/liter, respectively). Testing for adult susceptibility to deltamethrin and permethrin also was carried out according to the WHO method (1981b) and with standard WHO impregnated papers (i.e., 0.025% deltametrin and 0.25% permethrin). Specifically, batches of 15 females, 5 days old and fed on sugar solution, were exposed for 1 h to the impregnated papers and checked for mortality after 24 h. Tests were carried out in triplicate (about 45 females/insecticide/population). A long-established laboratory strain of *Ae. aegypti* (ISS) was used as a susceptible control for both larval and adult bioassays.

RESULTS

The results of the exposure of the 15 larval populations of *Ae. albopictus* to temephos are shown in Table 1. For the populations collected in 2002, the LC_{50} values ranged from 0.0026 to 0.0085 mg/liter, and the LC_{99} values ranged from 0.0093 to 0.023 mg/liter. All of the LC_{99} values obtained in 2002 were below the conventional diagnostic concentration of 0.02 mg/liter, suggested by WHO for discriminating between susceptibility and resistance of *Ae. aegypti* to temephos (WHO 1981a), with the exception of the populations from Abano and Brescia 1, which had LC_{99} values of 0.023 and 0.022 mg/liter, respectively. Nonetheless, when these 2 larval populations were tested directly with the diagnostic concentration, mortality was 100% (data not shown because not considered by the log-probit regression lines). All of the larval populations tested in 2002 also were susceptible to the diagnostic dosages of chlorpyrifos and fenthion (100% mortality to 0.01 and 0.05 mg/liter, respectively) suggested by WHO (1981a) for *Ae. aegypti.* The exposure to one half of these dosages also resulted in 100% mortality, with the following exceptions that showed survival rates comprised from 5 and 10%: Abano (≅ 5% survival to 0.005 mg/liter of chlorpyrifos), Padova (≅ 10% survival to 0.005 mg/liter of chlorpyrifos), Brescia 1 (≅ 10% survival to 0.005 mg/liter of chlorpyrifos), Lignano (≅ 20% survival to 0.005 mg/liter of chlorpyrifos and ≅ 10% to 0.025 mg/liter of fenthion), Savignano S.R. (≅ 10% survival to 0.025 mg/liter of fenthion), Rome 1 (≅ 10% survival to 0.005 mg/liter of chlorpyrifos), and Rome 2 (≅ 5% survival to 0.005 mg/liter of chlorpyrifos).

In 2002, adult females from all of the study sites were fully susceptible (100% mortality) to 1 h of exposure to the diagnostic dosages of permethrin (0.25%) and deltamethrin (0.025%).

Table 1. Susceptibility of larval populations of *Aedes albopictus* from Italy to temephos, 1992–2002.

City or town (province)[1]	Years of treatment	Lethal concentrations (LC_{50}/LC_{99})[2]			
		1992–93	1996	1998–99	2002
Genova 1 (west coast) (GE)	11	0.0026/0.0076	0.0025/0.0080	0.0024/0.0078	0.0030/0.012
Genova 2 (east coast) (GE)	11	—	—	—	0.0026/0.011
Padova 1 (downtown) (PD)	10	0.0026/0.0087	0.0031/0.0087	0.0049/0.016	0.0074/0.019
Padova 2 (E outskirts) (PD)	11	—	—	—	0.0078/0.017
Abano T. (PD)	10	—	0.0033/0.011	0.0038/0.011	0.0057/0.023
Lignano S. (UD)	2	—	—	—	0.0072/0.018
Brescia 1 (downtown) (BS)	10	0.0075/0.013	0.0079/0.015	0.0082/0.016	0.0084/0.022
Brescia 2 (outskirts) (BS)	10	—	—	—	0.0085/0.019
Bazzano (BO)	2	—	—	—	0.0043/0.010
S. Lazzaro di S. (BO)	2	—	—	—	0.0045/0.012
Casalecchio di R. (BO)	3	—	—	0.0031/0.012	0.0037/0.011
Savignano sul R. (FO)	6	—	—	—	0.0073/0.014
Grosseto (GR)	2	—	—	—	0.0052/0.0095
Roma 1 (NW side) (RM)	5	—	—	0.0048/0.013	0.0053/0.0093
Roma 2 (SE side) (RM)	5	—	—	—	0.0063/0.013
ISS (*Ae. aegypti*)[3]	—	0.0023/0.0066	0.0025/0.0074	0.0024/0.0065	0.0026/0.0077

[1] Provinces are abbreviated as follows: GE, Genova; PD, Padova; UD, Udine; BS, Brescia; BO, Bologna; FO, Forlì-Cesena; GR, Grosseto; RM, Roma.
[2] Concentrations listed in mg/liters.
[3] ISS, *Aedes aegypti* reference strain.

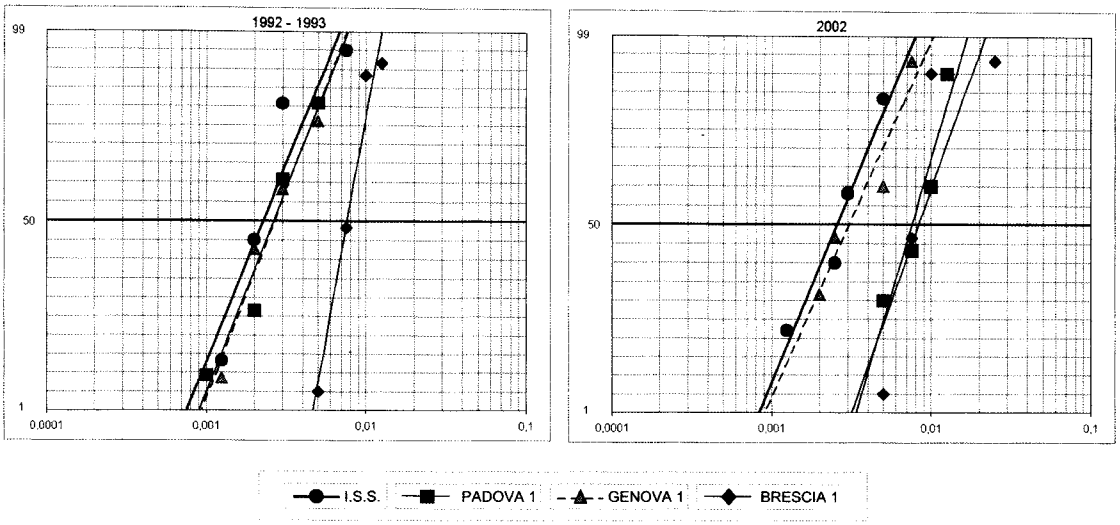

Fig. 2. Changes in the susceptibility to temephos of 3 larval populations of *Aedes albopictus* established in Italy since the early 1990s (Padova 1, Genova 1, and Brescia 1). Log-probit regression lines obtained in 1992–93 (left) are compared with those obtained in 2002 (right). A long-established laboratory strain of *Aedes aegypti* (ISS) was used as a susceptible control.

DISCUSSION

According to the data for 2002, most of the populations of *Ae. albopictus* considered in this study were still susceptible to temephos and to deltamethrin, the two most commonly used insecticides for larval and adult control of mosquitoes in Italy. However, when comparing the data on larval temephos susceptibility from 2002 to those for the 3 previous periods, it appears that the LC_{50} and LC_{99} values for the populations from Padova 1 and Brescia 1 have been progressively increasing and that in 2002, LC_{99} values of both populations were about 2-fold higher with respect to those of 1992 (Fig. 2). In these areas, larvicidal treatment with temephos, which has been carried out intensively for the past 10–11 years, may have induced a slow yet progressive decrease in susceptibility. Nonetheless, less evident changes in the susceptibility to temephos appear to have occurred in the population of *Ae. albopictus* from Genova 1 (Fig. 2) where, although the species was 1st reported there 12 years ago, the larvicidal treatments performed to date in this city have been sporadic and ineffective.

Relatively little information has been published on the susceptibility or resistance of *Ae. albopictus* to insecticides (Herbert and Perkins 1973, Robert and Olson 1989, Wesson 1990, Wu et al. 1992, Ali et al. 1995). Examination of our data on temephos indicates that the LC_{99} values for some Italian populations of *Ae. albopictus* are moving towards, or exceeding, the threshold of 0.02 mg/liter established by WHO for *Ae. aegypti.* However, according to some authors, larval *Ae. albopictus* are less susceptible than larval *Ae. aegypti* to organophosphate insecticides, and the diagnostic dosage of temephos and of the other organophosphates should be at least doubled (Wesson 1990). In light of these data, the Italian populations of *Ae. albopictus* considered in this study would be still fully susceptible to temephos and to other organophosphates. Moreover, considerable variation between geographic populations of *Ae. albopictus* and *Ae. aegypti,* in response to insecticide application, also has been reported (Wesson 1990), and studies carried out on field strains of *Ae. albopictus* from Asia and the United States have shown that temephos $LC_{90/95}$ values >0.02 mg/liter are very common (Wesson 1990, Wu et al. 1992, Ali et al. 1995).

Although some of the sites considered in this study have been treated for at least 10 years, we only observed a gradual decrease in susceptibility to temephos. This is perhaps because the treated larval breeding sites only represent a small proportion of the actual number of breeding sites present in a given area; thus, only a part of the larval population would be subjected to the selective pressure exerted by larvicides. Specifically, it is possible that the most commonly treated sites (i.e., street drains) are not always the most productive breeding sites. In fact, although street drains may be productive sources of larvae in the spring and fall (i.e., when rain is abundant), many of them often dry up in the warmest months of the year (July–September). In this case most of the breeding sites of *Ae. albopictus* could be represented by the vast number of containers scattered in the infested areas, where larvae may breed in absence of any insecticide treatment.

Additional studies will be needed to determine the contribution of street drains to the total population of *Ae. albopictus* and to monitor periodically the susceptibility to insecticides of populations of *Ae. albopictus* in Italy.

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