THE SUSCEPTIBILITY OF MOSQUITOES TO INSECTICIDES IN SALAMANCA PROVINCE, SPAIN

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ABSTRACT. Larval susceptibility to temephos, malathion and fenitrothion were studied in 6 *Aedes*, 2 *Culex* and one *Anopheles* species from areas with the following agricultural characteristics: VdT, intensively irrigated crops; Zone A, cereals and some irrigation; Zone B, cereals and grasslands; Zone C, oak and pine forests. The susceptibility of adult *Anopheles* from VdT and Zone B areas to dieldrin and DDT was also studied. The use of insecticides in these areas probably decreases from VdT, to Zone A, to Zone C, to Zone B. Tests of larval and adult susceptibility of mosquitoes in these areas followed the recommendations of WHO re materials and methods. In the absence of any baseline data concerning the susceptibility of the tested mosquito population, the test results only allow one to suspect changes in susceptibility to temephos, malathion and fenitrothion in larvae of *An. atroparvus* and *Cx. theileri* from VdT and resistance in adults of *An. atroparvus* to dieldrin and DDT.

INTRODUCTION

The growth of semiurban areas in the towns of Salamanca Province, in which rural activities and human settlements overlap, has led to increased interest in mosquitoes owing to their biting habits and their possible role as vectors of pathogens. Until now, mosquitoes in this province have been controlled only by the introduction of larvivorous fish. Anticipating the possible need of insecticides to control mosquitoes in Salamanca, we attempted to determine their susceptibility to some agents in common use.

In Central America, Georghiou (1972) found strong evidence that agricultural insecticides have selected for resistance, depending on the amount of insecticide used in agriculture. Therefore our study was designed at district level, since the differing crops cultivated in each district require widely varying amounts of insecticide for pest control.

As there were no provincial records of the types of insecticides employed each year or the areas in which they were used, we deduced the presumptive use of the insecticides in the different areas according to the kind of crops grown in them. Our choice of compounds for testing was guided not by the agricultural chemicals to which the mosquitoes had been exposed but by those which are most likely to be used for mosquito control in the future.

Species and study area (Fig. 1). Of the 32 species reported from Salamanca province (Encinas Grandes 1982), those selected for testing were the ones which were abundant and showed distribution patterns in the province coinciding with some of the natural divisions, which in turn differ in their patterns of land use.

In what follows we indicate the predominant crops of each district and the presumptive use of insecticides in them. With a view to highlighting the differences between them, we also provide data relating to their characteristic mosquitoes. Those marked with an asterisk were the ones chosen for our assays.

Zone A: La Armuña (except Vega del Tormes). *Aedes refiki* Medschid, *Aedes rusticus* (Rossi)*, *Aedes cantans* (Meigen), *Aedes excrucians* (Walker)*, *Aedes flavescens* (Muller), *Aedes sticticus* (Meigen), *Aedes leucomelas* (Meigen), *Anopheles claviger* (Meigen) and *Culiseta litorea* (Shute). Cultivation is mainly of non-irrigated cereals and use of insecticides is uncommon and limited to isolated irrigated areas.

Vega del Tormes Area (VdT). Enclave located in the previous zone but the dominant species are *Aedes caspius* (Pallas)*, *Aedes vexans* (Meigen)* and *Coquillettidia richiardii* (Ficalbi). Cultivation is mostly irrigated (maize, alfalfa, sugar beet, etc.) and the use of insecticides is greater than in any other zone.

Zone B: Central and western part of the province. The characteristic mosquito species are *Aedes quasirusticus* (Torres Cañamares)*, *Aedes vittatus* (Bigot), *Anopheles petragnani* Del Becchio and *Culiseta fumipennis* (Stephens). Grassland and non-irrigated cereals predominate and there is little or no use of pesticides except for control of ectoparasites on domestic ungulates, treatment of stables and control of the potato beetle on small holdings.

Zone C: Mountains of Béjar and Sierra de Francia. The characteristic mosquito species is *Aedes punctor* (Kirby)*. Oak and pine forests predominate and use of insecticides is sporadic for the treatment of pines and the fruit trees cultivated in the warmest areas.

Other species studied were *Anopheles atroparvus* Van Thiel, *Culex theileri* Theobald, and *Culex pipiens* Linn.; unlike the above mentioned species from zones A, B, C and VdT, these are common throughout all the areas specified. Of all the possible populations, our study concentrated on those occupying contrasting sites with respect to their putative contamination by insecticides, viz., the areas of intensive cultivation

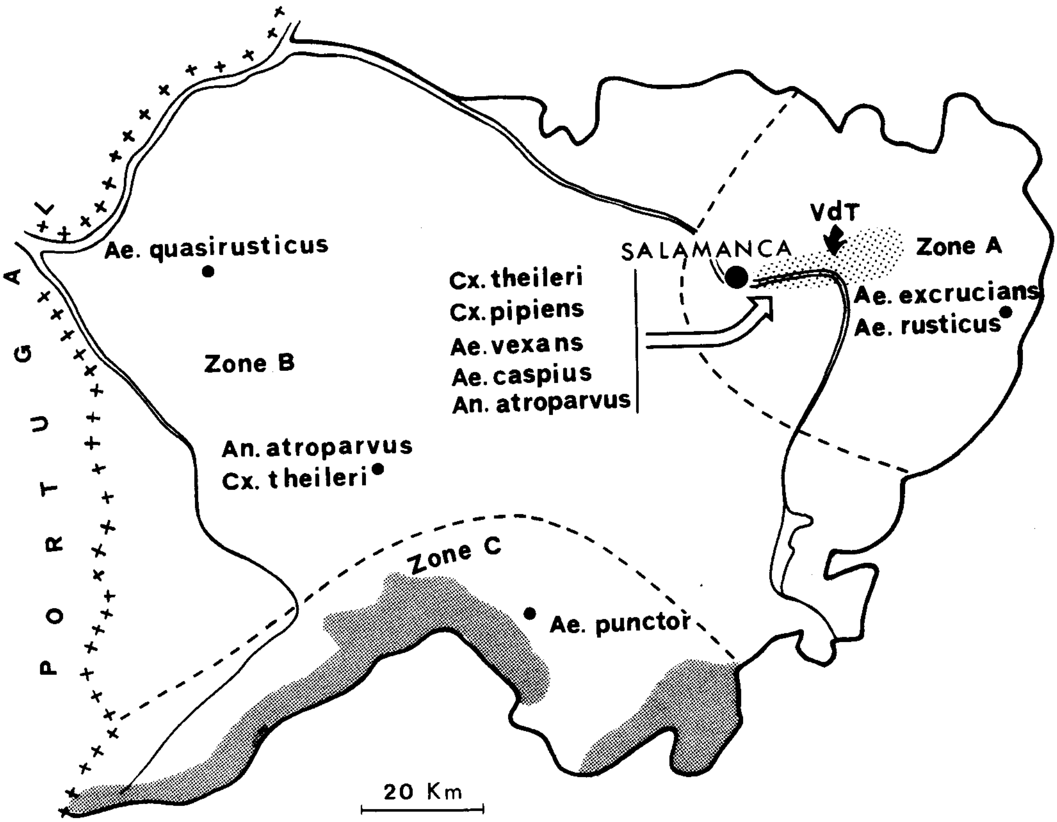

Fig. 1. Collection sites of the mosquitoes studied with respect to the natural divisions of Salamanca Province. The darker shaded areas represent the mountainous zones.

of VdT and the primarily pastoral area of Campocerrado (Zone B).

MATERIALS AND METHODS

Source of mosquitoes. All larvae were captured as 4th instars and were subjected to susceptibility tests within 24 h; until then they were kept in water from their collecting site. Adults of *Anopheles atroparvus* were obtained from 4th-instar larvae and pupae also taken from their natural breeding grounds. Until assay, 3–5 days postemergence, the adults were kept in insect cages at 25–30°C, 70–80% RH and 16 h light: 8 h darkness and were fed 5% glucose solution.

Insecticides. Susceptibility tests with larvae were conducted with 4 standard solutions of malathion, temephos and fenitrothion obtained from the World Health Organization (WHO), as was all necessary equipment (WHO 1981a). For adults tests, papers impregnated with dieldrin and DDT at diagnostic concentrations (0.4 and 4%, respectively) were used; these were also provided by the WHO, together with standard test kits (WHO 1981b).

Bioassays. Probit mortality/log dose regression lines were obtained (as described by Swaroop 1969) for larvae of all the species, with strict attention to WHO recommendations (WHO 1981a); for each insecticide 4 concentrations were tested with a total of 100 larvae per concentration.

Owing to the difficulty of obtaining a sufficient number of adults of *An. atroparvus*, study with these was limited to exposing the females at the diagnostic dosages and time specified by the WHO (1981b) and Brown (1983); no attempt was made to obtain dosage or time/mortality regression lines.

RESULTS

For the 3 OP insecticides (Table 1) the VdT larval populations of *An. atroparvus* and *Cx. theileri* showed LC values 2–3 fold higher than the populations of the same species from Zone B. In contrast, *Ae. caspius* and *Ae. vexans* from VdT had LC values similar to or lower than those obtained for other species of the genus, including *Ae. quasirusticus* which, owing to its origin, has presumably never undergone selection by insecticides. Finally, the larvae of *Cx.*

Table 1. Insecticide concentration/mortality responses of mosquitoes from Salamanca Province, Spain. Concentrations are given in ppm.

			Temephos			Fenitrothion			Malathion		
Species	Zone	Date	LC_{50}	$LC_{99.9}$	χ^2 (2 d.f.)	LC_{50}	$LC_{99.9}$	χ^2 (2 d.f.)	LC_{50}	$LC_{99.9}$	χ^2 (2 d.f.)
Anopheles atroparvus	VdT	Aug. 1984	0.0138	0.1210	5.62	0.0363	0.3410	13.84**	0.1510	1.3640	7.81*
Anopheles atroparvus	B	Aug. 1985	0.0047	0.0384	16.06***	0.0178	0.1270	9.34**	0.0833	0.6580	9.03*
Culex theileri	VdT	Aug. 1984	0.0058	0.0628	8.43	0.0154	0.1220	5.69	0.1540	1.6790	11.99**
Culex theileri	B	Sep. 1985	0.0019	0.0142	12.15**	0.0049	0.0324	10.13**	0.0485	0.3780	14.62***
Culex pipiens	VdT	Aug. 1984	0.0010	0.0063	2.62	0.0021	0.0133	12.14**	0.0845	0.4930	8.18*
Aedes vexans	VdT	May 1985	0.0020	0.0221	6.88*	0.0045	0.0321	4.35	0.0547	0.2990	3.96
Aedes caspius	VdT	May 1985	0.0013	0.0080	10.19**	0.0035	0.0197	5.65	0.0279	0.1720	4.20
Aedes excrucians	A	Mar. 1985	0.0012	0.0061	3.98	0.0053	0.0360	6.22*	0.0681	0.4970	14.60***
Aedes rusticus	A	Mar. 1985	0.0029	0.0252	23.83***	0.0079	0.0384	1.29	0.0863	0.3850	4.71
Aedes quasirusticus	B	Feb. 1985	0.0016	0.0074	3.43	0.0038	0.0182	4.71	0.0345	0.1890	7.54*
Aedes punctor	C	Apr. 1985	0.0021	0.0123	2.67	0.0070	0.0427	12.06**	0.0512	0.3260	2.86

* $0.05 > P > 0.01$.
** $0.01 > P > 0.001$.
*** $P < 0.001$.

pipiens captured at the same time and at sites near to those of *An. atroparvus* and *Cx. theileri* showed the highest susceptibility to 2 of the 3 insecticides tested.

One year after these tests, the mortalities recorded for larvae of *An. atroparvus* from VdT exposed to the WHO-recommended diagnostic dose were 100% with temephos and malathion, and 37% for fenitrothion. *Culex theileri* from the same area were tested with the diagnostic dosages of malathion and showed 50% mortality.

A small number of tests with adults of *An. atroparvus* (Table 2) showed low mortalities with dieldrin for the 2 populations (VdT and Zone B) studied. With DDT mortalities were higher but never reached 100%, even after 2 h of exposure.

DISCUSSION

In the absence of any baseline data concerning the susceptibility of the tested mosquito populations to temephos, fenitrothion and malathion, it is difficult to evaluate the test results since the LC values given by other authors and the diagnostic doses provided by the WHO are only orientative for the judgment of our results. Despite this difficulty, however, we consider that none of the *Aedes* species studied nor *Cx. pipiens* have lost any of their susceptibility since their LC values were equal to or less than those reported by Gras et al. (1966), Rettich (1977), Sinegre (1982) and Brown (1983) for populations of *Cx. pipiens* defined as being susceptible or for *Aedes* species the same as or closely related to our own.

In the *An. atroparvus* and *Cx. theileri* populations from VdT the situation may be different. The slight increase in the LC values of these populations compared with those of Zone B together with the survival of the larvae against some WHO diagnostic doses, although they may be due to other causes (Brown and Pal 1971), may also point to an incipient resistance triggered by the agricultural insecticides used in VdT, as has been observed in other areas (Brown 1983 and Georghiou 1983). The fact that the other species (*Ae. caspius*, *Ae. vexans* and *Cx. pipiens*) from VdT were not affected may be due, in the case of the *Aedes*, to a low number of generations which does not favor the rapid evolution of resistance (WHO 1976, Patton et al. 1982, Georghiou 1983) and to the fact that most of the populations there develop in spring, before the fields are sprayed with insecticides. The unchanged susceptibility of *Cx. pipiens* might be due to the crossing of populations subject to insecticide pressure with others that are not, such as those that develop in the peridomestic waters of VdT villages.

The small samples tested indicated resistance of adults of *An. atroparvus* to dieldrin and DDT. These compounds have been prohibited in Spain since 1975, and although the resistance of the VdT populations might be attributed to the long persistence of these agents in the environment, the same cannot be said for Zone B (Campocerrado) where there is no evidence that they have been used at any time. Sinegre et al. (1977) working with *Cx. pipiens*, and Beard et al. (1985)

Table 2. Twenty four hour mortality of female *Anopheles atroparvus* exposed to dieldrin (0.4%) or DDT (4%). Number of individuals tested shown in parentheses.

Insecticide	Zone (month of assay)	Exposure time: 1 hr	2 hr
Dieldrin	VdT (June)	0% (25)	—
	B (Aug.)	27% (11)	30% (10)
DDT	VdT (June)	29% (31)	—
	B (Aug.)	70% (10)	90% (10)

working with *Aedes aegypti* (Linn.), reported similar findings with organochlorine pesticides and also found it difficult to give a plausible explanation. In our case we believe that the use of these agents, together with HCH for control of ectoparasites on domestic ungulates and in their stables, is perhaps one of the causes. We consider this resistance in *An. atroparvus* to be important because previously this mosquito was the most widespread vector of malaria in Spain (Bruce-Chwatt and Zulueta 1980).

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