Journal of the American Mosquito Control Association, 15(3):308–311, 1999

RESISTANCE TO TWO PYRETHROIDS IN *ANOPHELES SINENSIS* FROM ZHEJIANG, CHINA

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ABSTRACT. Probabilities of pyrethroid resistant genotypes in natural populations of *Anopheles sinensis* Wiedemann were measured with deltamethrin and permethrin. The median lethal concentrations (LC_{50}s) of deltamethrin and permethrin in the susceptible larval population were 0.0209 and 0.1747 ppm, respectively. Under dosages that produced 99% mortality in susceptible laboratory strains of larvae, the lethal percentage of Cangnan larval field populations after 20 min of exposure was only 61.23% for deltamethrin and 64.92% for permethrin. This was much lower than those of other natural populations. Also, the probability of pyrethroid-resistant genotypes in Cangnan adult field populations was at the highest, reaching 0.5867. The results are discussed in relation to future mosquito control programs.

KEY WORDS *Anopheles sinensis,* pyrethroids, resistance, mosquitoes, bioassay

INTRODUCTION

In Zhejiang, organophosphate (OP) insecticides have been used to control mosquitoes for more than 20 years and OP resistance is widespread in most mosquito species (Wang et al. 1996b). Therefore, replacement of OP insecticides with other classes of insecticides, such as pyrethroids, is important for enhancing mosquito control.

Anopheles sinensis Wiedemann, a major vector of malaria and filariasis, is widely distributed in rural areas of Zhejiang, People's Republic of China. Pyrethroids have been used in cultivated lands to control crop pests since the early 1980s, but no data are available on the resistance of mosquitoes to pyrethroids. In 1991, mosquito-curtains treated with deltamethrin were used to control *An. sinensis* in the rural area of Cangnan, Zhejiang. This is the 1st report on mosquito control with pyrethroid-treated curtains in Zhejiang. This work was carried out for 2 years and resulted in a sharp decrease in malaria (Yao 1992).

In order to coordinate the research on mosquito-curtain treatments in Cangnan, we carried out research on pyrethroid resistance in mosquitoes to determine the pyrethroid resistance level in the Cangnan population of *An. sinensis* by comparing the lethal concentration of pyrethroids to that of a laboratory susceptible strain. Because pyrethroid resistance is now widespread in many crop pest species in Zhejiang (Zhai 1995), possible pyrethroid resistance of mosquito populations in other rural areas of Zhejiang was also investigated by conducting bioassays and comparing these natural populations with the Cangnan population and the susceptible laboratory strain.

MATERIALS AND METHODS

Mosquitoes: The susceptible strain of *An. sinensis* came from a laboratory population that had been without insecticide selection for 15 years. Natural populations of *An. sinensis* were collected from breeding sites in Cangnan, Jinhua, Yuyao, Hangzhou, and Jiaxing during the summer of 1996. The collected adults were kept in screen cages (29 × 22 × 23 cm) and fed 15% glucose solution. A silk fabric laid on moistened cotton in the culture plate was supplied for oviposition. Larvae were reared in an enamel bowl containing 200 ml of water under conditions described by Wang et al. (1996a). Pupae and adults were reared in the screen cages under similar conditions. The 4th-stage larvae and the female adults in the 2nd or 3rd Christophers' stage were used in the bioassays.

Insecticides: Deltamethrin (98% powder) and permethrin (95.45% emulsion) supplied by the Chemical Laboratory, Zhejiang Academy of Agricultural Sciences, Hangzhou, were diluted to 1% and 0.1% stock solutions with acetone.

Bioassay: Sensitivities of susceptible larvae to these 2 pyrethroids were measured in 200 ml of water following the World health organization (WHO) bioassay protocol (WHO 1975, Liu 1985). For the range-finding test, the concentration gradient points were 2 for deltamethrin and 2.5 for permethrin, and each regular test contained 6 or 7 concentrations with 3 replicates per concentration. Mortality at 10, 15, and 20 min posttreatment was recorded. Relative concentrations of acetone were used as controls.

Pyrethroid resistance of larvae in each natural population was measured with the dosage producing 99% mortality (LC_{99} = 0.2187 ppm for deltamethrin and 1.8537 ppm for permethrin). The same conditions were applied to susceptible larvae in the laboratory. Mortality at 5-min time intervals from 5–40 min was recorded.

Field detection of resistance in adult mosquitoes currently relies heavily on the use of predetermined discriminating dosages (concentration × exposure time) (WHO 1986, Hemingway 1995). The discriminating dosage for deltamethrin was deter-

Table 1. Lethal concentrations (ppm) of 2 pyrethroids to susceptible larvae.

Insecticide	Treatment time (min)	Lethal concentration (ppm)		Regression equation		
		LC_{50}	LC_{99}	Intercept	Slope	Coefficient
Deltamethrin	10	0.5677 a	9.7336 a	5.4635	1.8849	0.9489
	15	0.0610 b	0.8755 b	7.4424	2.0105	0.9839
	20	0.0209 bc	0.2187 bc	8.8334	2.2833	0.9795
Permethrin	10	4.3662 a	251.4161 a	4.1540	1.3216	0.9239
	15	0.5962 b	15.8911 b	5.3665	1.6316	0.9817
	20	0.1747 bc	1.8537 c	6.7184	2.2679	0.9730

[1] LC_{50}, median lethal concentration; LC_{99}, 99% lethal concentration. The LC_{50}s or LC_{99}s in 2 treatment times without the same lowercase letter are significantly different ($t \geq 3.543$, df = 4, $P < 0.05$).

mined as 0.01%/20 min in the People's Republic of China (Liu and Chen, unpublished data). Tests were undertaken using the WHO standard susceptibility test tube (WHO 1981a, 1981b). The 1% stock solution of deltamethrin was diluted to 0.01% in solvent (50 ml refined mineral oil + 100 ml ether). Xinghua no. 1 filter paper (16 × 12.5 cm) was coated with 2.14 ml of solution, and then the inner wall of the test tube was lined with the filter paper for testing. The same volume of solvent without the insecticide was used as the control. Twenty individuals were placed in the test section of each tube to contact the insecticide layer for 20 min before being moved into the recovery section of the tube (Liu and Chen 1979). Mortality after 20 min and 24 h was recorded. Each population had 4 or 5 replicates.

Statistical analysis: The LC_{50} and LC_{99} values of each pyrethroid to susceptible larvae were calculated by linear regression. Resistance differences among mosquito populations were analyzed with Student's *t*-test.

RESULTS

Sensitivity of susceptible larvae

This experiment was undertaken to provide the standard dosages of pyrethroids for measuring resistance levels in natural mosquito populations. Lethal concentrations (LC_{50} and LC_{99}) of deltamethrin and permethrin to susceptible larvae are shown in Table 1. The LC_{50} and LC_{99} in 20 min were 0.0209 and 0.2187 ppm, respectively, for deltamethrin and 0.1747 ppm and 1.8537 ppm, respectively, for permethrin.

Parallelism of the mortality lines obtained at 10 min and the mortality lines obtained at 15 min was rejected at the 5% confidence level for the pyrethroids. The LC_{50} and LC_{99} values of the pyrethroids in 10 min were significantly ($t \geq 3.543$, df = 4, $P < 0.05$) higher than those in 15 min (9.3-fold and 11.1-fold for deltamethrin, respectively, and 7.3-fold and 15.8-fold for permethrin, respectively). This indicates that the treatment time had an important effect on the lethal rate in a shorter time range, but this effect would become significant if the treatment time were protracted further.

Probabilities of pyrethroid-resistant larval individuals in natural populations

Probabilities of pyrethroid-resistant larval individuals in natural populations were measured with the 2 pyrethroids producing 99% mortality in susceptible larvae (Table 2). The larval population resistance in Cangnan, where deltamethrin had been used for 5 years since 1990 for treating mosquito-curtains, was not only higher than that of susceptible larvae but also higher than those of larvae from other natural populations ($t \geq 5.007$, df = 4, $P < 0.01$). Parallelisms of the mortality lines obtained with 4 other natural populations were not rejected at the 5% confidence level for the pyrethroids, indicating no significant ($tr \geq 2.361$, df = 4, $P > 0.05$) difference in susceptibility of these natural larval populations.

Table 2. Mortality (%) in 20 min and medial lethal time (LT_{50}) of larvae.[1]

Population	Deltamethrin		Permethrin	
	Mortality (%)	LT_{50} (min)	Mortality (%)	LT_{50} (min)
Cangnan	61.23 ± 4.32*	17.82 ± 2.71*	64.92 ± 5.10*	18.11 ± 1.82*
Jinhua	90.79 ± 5.12	10.67 ± 2.11	94.82 ± 4.91	9.84 ± 1.23
Yuyao	97.40 ± 4.71	8.53 ± 1.85	90.20 ± 3.33	12.39 ± 2.01
Hangzhou	94.58 ± 3.89	12.96 ± 1.77	97.14 ± 4.87	12.19 ± 1.75
Jiaxing	91.26 ± 5.55	11.01 ± 1.24	93.51 ± 3.79	10.78 ± 1.09

[1] An * indicates a significant difference between the Cangnan population and each other natural population ($t \geq 5.007$, df = 4, $P < 0.01$).

Table 3. Mortality (%) in natural and susceptible adult populations.[1]

Population	20 min	24 h
Susceptible	88.53 ± 5.14	97.60 ± 6.10
Cangnan	41.33 ± 3.33**[a]	58.06 ± 5.55**
Jinhua	57.64 ± 2.51*	81.77 ± 3.49*
Hangzhou	64.65 ± 4.14*	89.74 ± 4.22*
Yuyao	60.23 ± 3.79*	90.85 ± 6.01*
Jiaxing	65.17 ± 2.98*	91.22 ± 5.77*

[1] Asterisks indicate a significant difference between each natural population and the susceptible population at * $P < 0.05$ ($t \geq 3.019$, df = 7) and ** $P < 0.01$ ($t \geq 3.994$, df = 7).

Median lethal times (LT_{50}s) of 2 pyrethroids to the larval population in Cangnan were significantly different from those of larval populations in other areas ($P < 0.05$), indicating that the use of pyrethroids to control mosquitoes resulted in the synchronous increases of resistance level and tolerance time.

Probability of adult resistance in natural populations

Adult resistance in each natural population was measured with the standard discriminating dosage (0.01%/20 min), and the adult resistance in a susceptible population was used as a control. The probability of deltamethrin-resistant adult individuals in the Cangnan population was at the highest level (Table 3). The percentages of resistant individuals in 20 min and 24 h were about 47 and 40% higher, respectively, than those of the susceptible population ($t \geq 3.994$, df = 7, $P < 0.01$). Probabilities of resistant individuals in other natural populations were also significantly different from those of the susceptible population ($t \geq 3.019$, df = 7, $P < 0.05$).

DISCUSSION

Mosquito-curtains treated with deltamethrin in Cangnan played an important role in controlling *An. sinensis* populations and consequently, the incidence of malaria in this area decreased greatly (Yao 1992). However, this research shows that continuous use of deltamethrin has caused an increase in pyrethroid-resistant individuals in the natural population.

Although only deltamethrin has been used in Cangnan to treat mosquito-curtains, permethrin resistance of mosquitoes was also detected, indicating that cross-resistance had occurred. However, effects on mosquitoes from agricultural uses of permethrin could not be ruled out. We inferred, but did not prove, that the cross-resistance of mosquitoes in Cangnan to other pyrethroids may have developed from deltamethrin usage.

For decreasing resistance and increasing the efficacy of insecticides on mosquitoes, we recommend the rotational use of different classes of insecticides. This method has met with great success in the control of the lepidopterans *Plutella xylostella* (L.) and *Helicoverpa armigera* (Hubner) (Hu and Wang 1997, Yan et al. 1997). In Cangnan, pyrethroids can be replaced with another class of insecticides or a synergist can be added to increase the effectiveness of the insecticides. For populations with low pyrethroid resistance levels and high OP resistance levels, such as those in Jinhua, Hangzhou, Yuyao, and Jiaxing (Wang et al. 1996b), pyrethroids are recommended for use, but with regular monitoring for the development of resistance to pyrethroids in those natural populations.

ACKNOWLEDGMENTS

We thank the epidemic prevention stations in Cangnan, Hangzhou, Yuyao, Jiaxing, and Jinhua for their help in the collection of mosquitoes. The present work was supported by the Zhejiang Foundation of Natural Sciences.

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