TOXICITY OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* TO MOSQUITO LARVAE VARIOUSLY RESISTANT TO CONVENTIONAL INSECTICIDES[1]

CHIH-NING SUN[2], GEORGE P. GEORGHIOU AND KATHY WEISS

Division of Toxicology and Physiology, Department of Entomology, University of California, Riverside, CA 92521

ABSTRACT. Two strains of *Anopheles albimanus* Wied. and five strains of *Culex quinquefasciatus* Say representing the principal mechanisms of resistance to organophosphorus, carbamate, DDT, dieldrin and pyrethroid insecticides were tested for cross-resistance to *Bacillus thuringiensis* var. *israelensis* toxin. Maximum interstrain differences in LC_{95} upon 48-hr exposure of 4th instar larvae were 1.9x for *Culex* and 1.5x for *Anopheles*, indicating that presently-existing resistance to insecticides should not significantly affect the larvicidal activity of this bacterial toxin.

The isolation of a new variant of *Bacillus thuringiensis* Berliner (ONR 60/WHO 1897) that shows high larvicidal activity (Goldberg and Margalit 1977) has opened new perspectives to the application of bacterial insecticides for mosquito control. This variant was subsequently identified by de Barjac (1978) as a new serotype, H_{14}, and designated as *Bacillus thuringiensis* var. *israelensis*. It has been reported to be highly toxic against at least 15 species in 7 genera of mosquitoes (Goldberg and Margalit 1977, Garcia and Desrochers 1979, de Barjac and Coz 1979), and against black fly larvae, especially *Simulium damnosum* s.l. (Guillet and de Barjac 1979).

In the present study, the possibility of cross-resistance to *B. t. israelensis* in strains of *Culex quinquefasciatus* Say and *Anopheles albimanus* Wied. that are resistant to conventional chemical insecticides has been examined. Two separate studies were performed: in the first, larvae were tested in the 2nd instar while in the second study they were tested in the 4th. The second study also included a time-course investigation of toxicity.

MATERIALS AND METHODS

B. t. israelensis was supplied by the Institut Pasteur, Paris, as standard powder (IPS 78) with 1000 international toxic units/mg. The species and strains of mosquitoes that were examined, their levels of resistance (resistance ratios at LC_{50}), the respective selection agent, and the principle mechanisms of resistance are indicated in Table 1.

The method BL-E, suggested by the Institut Pasteur for a biotest of IPS 78 (Anonymous 1979), was adopted for the first study and was modified for the second. A 4% (w/v) primary suspension of *B. t. israelensis* (4×10^4 international toxic units/ml) was prepared in distilled water and mixed with a Vortex mixer. Ten-fold serial dilutions were made. Distilled water and appropriate amounts of the suspension of *B. t. israelensis* were placed in plastic cups of 250 ml capacity. The testing solution totaled 150 ml with dilutions of *B. t. israelensis* ranging from 10^{-5} to 10^{-7}. Twenty-five 2nd instar larvae were placed in each cup and were provided with 30 mg of food per cup. *Cx. quinquefascitatus* received a mixture of ground lab chow and powdered yeast at a ratio of 3:1, and *An. albimanus* received powdered yeast only. The cups were kept at 26°C and ca. 60% R.H. Mortality counts were taken 24

[1] This investigation received financial support from the UNDP-World Bank-WHO Special Program for Research and Training in Tropical Diseases, and from Special State Funds for Mosquito Control Research appropriated annually by the California Legislature.

[2] Present address: Department of Entomology, National Chung-Hsing University, P.O. Box 17–27, Taichung, Taiwan 400, Republic of China.

and 48 hr later. Distilled water was added to the cups at 24 hr to compensate for loss due to evaporation. After the effective range of the toxicant had been established for each strain, 4–6 concentrations were chosen within the range producing 5%-95% mortality for the determination of dose-response regression lines (ld-p lines). Each concentration was evaluated on 3–4 different days and with at least 2 replications in each day.

In the second study, waxed paper cups, a final volume of 100 ml, and early 4th instar larvae were used. All other aspects of the procedure were the same as described above. In addition to the bioassay tests, a time-course study was made using the susceptible strain of *Cx. quinquefasciatus* Say to determine the length of exposure required for attainment of a steady state in toxic effects.

RESULTS AND DISCUSSION

The time-course study on 4th instar larvae indicated that toxic effects are produced within minutes following initiation of exposure and that a steady state is reached within approximately 24 hr (Fig. 1). Within 15 min of treatment, a concentration of 14 IU/ml (14 μg/ml) killed 50% of the larvae, while a concentration of 24 IU/ml produced 95% mortality within the same period. LC_{50} and LC_{95} values decreased sharply with time during the first 8 hr of exposure, but only small changes occurred beyond 24 hr.

LC_{50} and LC_{95} data for 2nd instar and 4th instar larvae (Tables 2,3) do not indicate the presence of significant differences in the response to *B. t. israelensis* toxin between insecticide-susceptible and -resistant strains of the species tested. The

Table 1. Insecticide resistance in strains examined for possible cross resistance to *Bacillus thuringiensis* var. *israelensis*.

Species and strain	Insecticide	resistance ratio[a]	Principal mechanism of resistance	References
Culex quinquefasciatus				
Susceptible		1x		
Propoxur-R	**Propoxur**	25x	oxidases	Georghiou et al. (1966)
	Temephos	2.9x		
	t-Permethrin	2.8x		
	DDT	67x		
Temephos-R	**Temephos**	320x	esterases	Ranasinghe and Georghiou (1980)
	Propoxur	2.7x		
	t-Permethrin	0.9x		
	DDT	19x		
trans-Permethrin-R	***t*-Permethrin**	4100x	nerve insensitivity	Priester and Georghiou (1980)
	DDT	1600x		
	Propoxur	4x		
	Temephos	1.4x		
cis-Permethrin-R	***c*-Permethrin**	450x	nerve insensitivity	Priester and Georghiou (1980)
	DDT	2400x		
	Propoxur	3.6x		
	Temephos	1.3x		
Anopheles albimanus				
Susceptible		1x		
OP/Carb.-R	**Propoxur**	>1000x	acetylcholinesterase (AChE) insensitivity	Ayad and Georghiou (1975); Ariaratnam and Georghiou (1974)
	Parathion	84x		
	Temephos	1x		
	t-Permethrin	0.76x		
	DDT	8x		
	Dieldrin	290x		

[a] Name of selecting insecticide indicated in bold face.

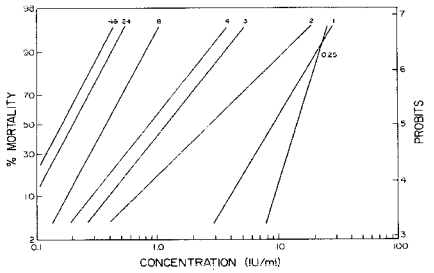

Fig. 1. Dose-response lines for 4th instar larvae of *Culex quinquefasciatus* exposed for varying lengths of time to *Bacillus thuringiensis* var. *israelensis* toxin. Numbers indicate hours of exposure. Results were evaluated at end of exposure period.

maximum interstrain differences at the LC_{95} were e.4x and 1.9x, respectively, in 2nd and 4th instar larvae of *Cx. quinquefasciatus*, and 1.8x and 1.5x, respectively, in 2nd and 4th instar larvae of *An. albimanus*. Thus, the existing mechanisms of resistance toward carbamates (oxidase detoxication or AChE insensitivity), organophosphates (esterase detoxication or AChE insensitivity), and pyrethroids (nerve insensitivity) do not appear to confer a significant advantage to mosquito larvae in the presence of *B. t. israelensis* toxin. Likewise, the cross-resistance to DDT or dieldrin that is present in certain of the strains tested, does not appear to extend to *B. t. israelensis*.

Anopheles was found to be more tolerant to *B. t. israelensis* than was *Culex*, in agreement with results reported by Goldberg and Margalit (1977), and de Barjac and Coz (1979). While there could be intrinsic differences in the susceptibility of these 2 species, it is also possible that the observed differences are due to the

Table 2. Susceptibility to *B. thuringiensis* var. *israelensis* of 2nd instar larvae of various strains of mosquitoes.

Species and strain	LC_{50} (μg/ml)[a] 24 hr	LC_{50} (μg/ml)[a] 48 hr	LC_{95} (μg/ml) 24 hr	LC_{95} (μg/ml) 48 hr
Cx. quinquefasciatus				
Susceptible	0.008 (0.006–0.013)[b]	0.005 (0.002–0.009)	0.039	0.036
RR[c]	[1.0]	[1.0]	[1.0]	[1.0]
Propoxur-R	0.012 (0.01–0.014)	0.008 (0.007–0.009)	0.044	0.029
RR	[1.5]	[1.6]	[1.1]	[0.81]
Temephos-R	0.009 (0.004–0.013)	0.006	0.036	0.017
RR	[1.1]	[1.2]	[0.92]	[0.47]
t-Permethrin-R	0.015 (0.012–0.019)	0.012 (0.009–0.016)	0.056	0.041
RR	[1.9]	[2.4]	[1.4]	[1.1]
c-Permethrin-R	0.018 (0.009–0.028)	0.013 (0.004–0.022)	0.046	0.034
RR	[2.3]	[2.6]	[1.2]	[0.94]
An. albimanus				
Susceptible	0.063 (0.045–0.082	0.042 (0.032–0.052)	0.42	0.16
RR	[1.0]	[1.0]	[1.0]	[1.0]
Parathion-R	0.068 (0.037–0.11)	0.049 (0.021–0.086)	0.41	0.29
RR	[1.1]	[1.2]	[0.98]	[1.8]

[a] μg/ml = IU/ml.
[b] Fiducial limits.
[c] Resistance Ratio = LC_{50} R strain/LC_{50} S strain.

Table 3. Susceptibility to *B. thuringiensis* var. *israelensis* of 4th instar larvae of various strains of mosquitoes.

Species and strain	LC_{50} (μg/ml)[a] 24 hr	LC_{50} (μg/ml)[a] 48 hr	LC_{95} (μg/ml) 24 hr	LC_{95} (μg/ml) 48 hr
Cx. quinquefasciatus				
Susceptible	0.18 (0.16–0.2)[b]	0.15 (0.13–0.17)	0.42	0.42
RR[c]	[1.0]	[1.0]	[1.0]	[1.0]
Propoxur-R	0.14 (0.12–0.16)	0.13 (0.09–0.15)	0.28	0.26
RR	[0.78]	[0.87]	[0.67]	[0.62]
Temephos-R	0.09 (0.07–0.13)	0.07 (0.06–0.09)	0.39	0.31
RR	[0.5]	[0.47]	[0.93]	[0.74]
t-Permethrin-R	0.19 (0.18–0.21)	0.16 (0.12–0.2)	0.54	0.50
RR	[1.1]	[0.89]	[1.3]	[1.2]
c-Permethrin-R	0.15 (0.11–0.18)	0.12 (0.08–0.15)	0.38	0.37
RR	[0.83]	[0.80]	[0.90]	[0.88]
An. albimanus				
Susceptible	1.3 (1.2–1.4)	0.86 (0.73–0.99)	4.4	2.6
RR	[1.0]	[1.0]	[1.0]	[1.0]
Parathion-R	1.8 (1.7–1.9)	1.0 (0.58–1.6)	4.9	3.9
RR	[1.4]	[1.2]	[1.1]	[1.5]

[a] μg/ml = IU/ml.
[b] Fiducial limits.
[c] Resistance Ratio = LC_{50} R strain/LC_{50} S strain.

feeding behavior of the species. Thus, *An. albimanus*, being a surface feeder, might have less access to the toxin than *Cx. quinquefasciatus* since the suspension tends to sink to the bottom of the container. In an attempt to determine if this was responsible for the observed difference in tolerance to *B. t. israelensis*, *An. albimanus* and *Cx. quinquefasciatus* were tested in water depths of 1 cm and 5 cm. Surprisingly, higher mortality was observed in both species with deeper water. In either case, *An. albimanus* was still more tolerant than *Cx. quinquefasciatus*. These results lead us to suspect that intrinsic factors or other aspects of feeding behavior may be responsible for the observed differences in susceptibility.

The evidence presented here that strains of mosquitoes resistant to conventional insecticides are as susceptible to *B. t. israelensis* as non-resistant strains provides support for the future utilization of this bacterial insecticide in mosquito control. In view of its distinctly different mode of action, this product also affords the opportunity for relaxation of selection pressure when it is included in a rotational spray program that utilizes bacterial as well as synthetic insecticides.

The slopes of the ld-p lines (Fig. 2) indicate that some of the strains show greater heterogeneity in their response to the toxin than is normally observed with conventional insecticides. Relatively low slopes are usually interpreted as indicating a potential for resistance in the test population. This may not be the case with *B. t. israelensis* toxin, however, in view of the unique mode of action of this product. Special studies are in progress that could provide answers to this question.

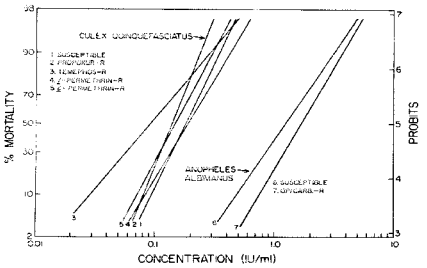

Fig. 2. Comparative susceptibility of 4th instar larvae of various strains of *Culex quinquefasciatus* and *Anopheles albimanus* to *Bacillus thuringiensis* var. *israelensis* toxin upon 24-hr exposure.

References Cited

Anonymous. 1979. Biotest of IPS 78 (Pasteur method BL-E). Institut Pasteur, Paris. mimeo: 3 pp.

Ariaratnam, V. and G. P. Georghiou. 1974. Carbamate resistance in *Anopheles albimanus*. Cross resistance spectrum and stability of resistance. Bull. WHO 51:655–59.

Ayad, H. and G. P. Georghiou. 1975. Resistance to organophosphates and carbamates in *Anopheles albimanus* based on reduced sensitivity of acetylcholinesterase. J. Econ. Entomol. 68:295–7.

de Barjac, H. 1978. Un nouveau candidat a la lutte biologique contre les moustiques: *Bacillus thuringiensis* var. *israelensis*. Entomophaga 23:309–19.

de Barjac, H. and J. Coz. 1979. Sensibilité comparée de six espèces différentes de moustiques *à Bacillus thuringiensis* var. *israelensis*. Bull. WHO 57:139–41.

Garcia, R. and B. Desrochers. 1979. Toxicity of *Bacillus thuringiensis* var. *israelenis* to some California mosquitoes under different conditions. Mosquito News 39:541–4.

Georghiou, G. P., R. L. Metcalf and F. E. Gidden. 1966. Carbamate-resistance in mosquitoes. Selection of *Culex pipiens fatigans* Wiedemann for resistance to Baygon. Bull. WHO 35:691–708.

Goldberg, L. J. and J. Margalit. 1977. A bacterial spore demonstrating rapid larvicidal activity against *Anopheles sergentii, Uranotaenia unguiculata, Culex univittatus, Aedes aegypti* and *Culex pipiens*. Mosquito News 37:355–8.

Guillet, P., and H. de Barjac. 1979. Toxicité de *Bacillus thuringiensis* var. *israelensis* pour les larves de Simulies vectrices de l' onchocercose. C. R. Acad. Sc. Paris, 289 (Série D): 549–52.

Priester, T. M. and G. P. Georghiou. 1980. Cross-resistance spectrum in pyrethroid resistant *Culex quinquefasciatus*. Pestic. Sci. (in press).

Ranasinghe, L. E. and G. P. Georghiou. 1979. Comparative modification of insecticide-resistance spectrum of *Culex pipiens fatigans* Wied. by selection with temephos and temephos/synergist combinations. Pestic. Sci. 10:502–508.