INVESTIGATIONS ON POSSIBLE RESISTANCE IN *AEDES VEXANS* FIELD POPULATIONS AFTER A 10-YEAR APPLICATION OF *BACILLUS THURINGIENSIS ISRAELENSIS*

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ABSTRACT. In the Upper Rhine Valley (Germany), *Bacillus thuringiensis* var. *israelensis* has been widely used against floodwater mosquitoes over an area of approximately 500 km^2 for more than 10 years. The susceptibility of larvae of *Aedes vexans* field populations in 3 untreated (Lake Constance) and 3 treated areas (Upper Rhine Valley) was assessed by means of bioassays with *B.t.i.* (Bactimos WP, 6,000 AAU/mg), following WHO guidelines. Log-probit analyses and statistical evaluations of the data showed that the LC_{50} values as well as slopes of bioassays of the larvae deriving from the different areas showed no significant differences. Two populations in the treated area were even more susceptible than populations from the untreated areas. These results have been confirmed by resistance ratios, which were less than one in all tests carried out.

INTRODUCTION

In the Upper Rhine Valley floodwater mosquitoes play an important role as a nuisance and can significantly reduce the quality of life of the residents. The most abundant species is *Aedes vexans* (Meigen), which forms more than 90% of the mosquito population during summer (Becker and Ludwig 1983). In response to this nuisance, 89 communities on both sides of the Rhine River merged their common interest into a united mosquito control program, the German Mosquito Control Association (KABS).

In an integrated control program, *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) is by far the most widely used biological control agent. Up to 1992, 45 tons of fluid and powder formulations were successfully applied to more than 50,000 ha and thus brought about a substantial reduction of the mosquito population in a control area of approximately 500 km^2.

More attention has been recently paid to the resistance phenomena since *Plutella xylostella* showed a high level of resistance to *B.t. kurstaki*, which is specific for lepidopterans (Tabashnik et al. 1990). Until now, data on mosquito resistance have been available only from laboratory studies. The results of Georghiou et al. (1983), Vasquez-Garcia (1983),[1] and Gharib and Szalay-Marzso (1986) do not indicate a significant decrease in susceptibility in different mosquito species.

After more than 10 years of *B.t.i.* applications in the Upper Rhine Valley, we decided to investigate if resistance had occurred under the sustained selection pressure of *B.t.i.* treatments. This study is based on a comparison of the susceptibility of *Aedes vexans* populations obtained from selected untreated areas (Lake Constance) and treated areas (Upper Rhine Valley), which are 300 km apart.

MATERIALS AND METHODS

Soil samples containing eggs of *Aedes vexans* were collected within the following areas: A) untreated area: 3 separate sites (1, 2, 3) near Lake Constance, and B) treated area: 3 separate sites (4, 5, 6) within the Upper Rhine Valley. Within the larval habitats, 1 m^2 soil samples from the upper layer (approximately 1 cm deep) were removed with a trowel and brought to the laboratory. The samples were kept for 14 days at 25°C to ensure conditioning of the eggs (Becker 1989, Becker and Ludwig 1981). After this period the soil samples were flooded in plastic vessels (40 × 40 × 20 cm) with a water layer up to about 20 cm above the soil. The hatched larvae were reared at 25°C and fed with fish food (Tetramin). All bioassays were conducted with late third and early fourth instar larvae.

The bioassays were done according to World Health Organization guidelines (WHO 1981): as follows: 50 mg *B.t.i.* (Bactimos WP, 6,000 AAU/mg, Novo Nordisk, Denmark) were added to 10 ml of distilled water and homogenized in a mixing machine (IKA Combimag Reo) at 700 rpm for 10 min, then homogenized in an ultrasonic bath (Branson Instruments) for 15 minutes. One ml was taken from the homogenized solution and added to 99 ml of distilled water. Depending on the concentration required, a range of 15 to 1,500 µl of homogenized and diluted Bactimos WP suspension was added to 200 ml plastic cups, which had been previously filled with 148 ml of

[1] Vasquez-Garcia, M. 1983. Investigations of the potentiality of resistance to *Bacillus thuringiensis* ser. H-14 in *Culex quinquefasciatus* through accelerated selection pressure in the laboratory. Ph.D. Dissertation, University of California, Riverside.

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Table 1. LC_{50}, confidence interval, slope and resistance ratio for all groups.

Group	LC_{50}[a]	95% C.I.* Lower	Upper	Slope	RR[b]
Untreated 1	0.126ab	0.118	0.134	6.8 ± 1.3a	
2	0.132a	0.123	0.141	6.0 ± 1.6ab	
3	0.099cde	0.085	0.114	3.7 ± 0.6cde	
$\bar{x}$ (untreated)	0.119 ± 0.017			5.5 ± 1.6	
Treated 4	0.107bcd	0.097	0.118	4.2 ± 0.7bcde	0.9
5	0.109bc	0.098	0.119	4.3 ± 0.4bcd	0.9
6	0.100cde	0.092	0.110	5.2 ± 0.6abc	0.8
$\bar{x}$ (treated)	0.105 ± 0.011			4.6 ± 0.5	0.9

* Values reflect the average of 3 replicates.

[a] µg/liter at 48 h.

[b] Resistance ratio (LC_{50} of the groups of the *B.t.i.*-treated areas/$\bar{x}$ of LC_{50} of the runs of untreated areas).

Values under the LC_{50} level and under the slope level followed by the same letter are not significantly different ($P \leq 0.05$).

distilled water. To each cup 25 larvae of *Aedes vexans* were added in 2 ml of water. Tests were run at 6 different concentrations with controls in 3 replicates per sampling site.

The mortality rate was evaluated after 24 and 48 h and corrected according to Abbott's formula (Abbott 1925). The results were subjected to log-probit analysis (Finney 1971, Raymond 1985) and data were treated by Duncan's multiple range test and Student's *t*-test (Köhler et al. 1984).

RESULTS

The LC_{50}-values as well as slopes (Table 1) of bioassays conducted with larvae deriving from the areas showed no significant differences except for areas 1 and 2 (tested by Duncan's multiple range test). The LC_{50}-values and slopes of these 2 groups were even higher than the LC_{50}-values of groups with larvae of the *B.t.i.* treated areas. These results have been confirmed by resistance ratios (Table 1), which are less than 1 in all groups.

Figure 1 shows mean values ± standard deviation of the LC_{50} and LC_{90} of the bioassays conducted with larvae from all 6 investigated areas. Student's *t*-test (Köhler et al. 1984) showed that there were no significant differences between the means of the LC_{50} and LC_{90} values, as well as between the means of the slopes of the bioassays with *Aedes vexans* originating from treated and untreated areas.

Figure 2 compares the log-probit lines of bioassays conducted with specimens of *Aedes vexans* originating from untreated and treated areas. The similarity (parallelism) of the lines also shows that no resistance phenomena have yet developed in the areas of the Upper Rhine Valley treated with *B.t.i.*

DISCUSSION

The rapid development of resistance is one of the major problems in the control of insects with chemical insecticides. In contrast to this, the possibility of the rapid development of resistance against microbial control agents seems to be unlikely to the same extent as the complex mode of action between pathogens and target organisms increases (Davidson 1992). Nevertheless, resistance against microbial insecticides is possible in principle. The investigations of Tabashnik et al. (1990) have shown that routine treatments with *B.t. kurstaki* (*B.t.k.*) products in agriculture can lead to a significant resistance within a few years.

Only a small number of papers deal with the resistance phenomena against *B.t.i.* among mosquito populations, and the results do not provide significant evidence of a permanent resistance in all cases. For example, Vasquez-Garcia

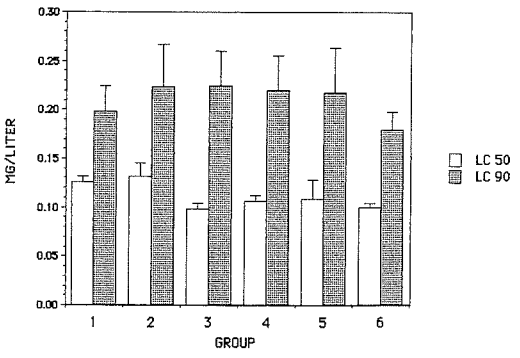

Fig. 1. Mean values ± standard deviation of the LC_{50} and LC_{90} values of the bioassays with *Aedes vexans* deriving from 6 areas (1–3 = *B.t.i.* untreated areas, 4–6 = *B.t.i.* treated areas).

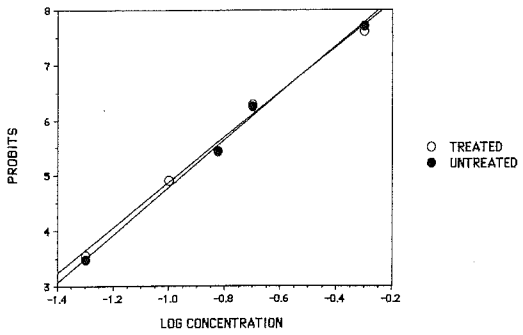

Fig. 2. Comparison of the log-probit-lines of the bioassays with *Aedes vexans* originating from untreated and treated areas. Data from sites 1–3 and 4–6 were pooled.

(1983)[1] treated laboratory populations of *Culex quinquefasciatus* Say with *B.t.i.* at varying levels of selection over 32 generations and found only a 5–7 fold decrease in susceptibility. This resistance phenomenon almost completely disappeared after a period of 3 generations without selection pressure. Goldman et al. (1986) found a 2-fold increase of the resistance ratios in only one out of 3 populations of *Aedes aegypti* (Linn.) following 14 generations of selection (LC_{50}) with *B.t.i.* Gharib and Szalay-Marzso (1986) demonstrated that a 1.9 fold increase of the LC_{50} values took place when 25 generations were under selection pressure. Georghiou et al. (1983) using a higher selection pressure (LC_{95}), reached an 11-fold decrease of the *Culex quinquefasciatus* susceptibility after 32 generations.

No data are available about the resistance phenomena of *B.t.i.* treated mosquito field populations. Nevertheless Kurtak et al. (1989) found no significant increase of resistance in *Simulium damnosum* populations after 7 years of extensive black fly control with *B.t.i.* in the Onchocerciasis Control Programme.

There may be several reasons why resistance was not found in *Aedes vexans*, in spite of more than 10 years of extensive control of floodwater mosquitoes with *B.t.i.*: 1) The rather short exposure period of the toxins. The confrontation between the toxin and the target organisms takes place for only a short time after application. 2) The unique and complex *B.t.i.* mode of action. It is assumed that the lethal changes within the cells of the midgut are produced by the synergistic effects of different proteins of the parasporal body (Federici et al. 1990). 3) Variable gene pools within target populations. *Aedes vexans* migrates within the breeding areas. This behavior leads to a constant gene flow, which at least delays the development of resistance. The phased eclosion of *Aedes vexans* produces generations that are not homogeneous, which also leads to an increased gene pool within the populations (Becker 1989).

Although no evidence of resistance to *B.t.i.* was found in the *Aedes vexans* populations of the Upper Rhine Valley, the susceptibility of the mosquito populations should be investigated at least every 3 years.

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