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EXPERIENCES WITH THE USE OF *BACILLUS THURINGIENSIS* SUBSP. *ISRAELENSIS* IN HUNGARY AT LAKE BALATON*

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ABSTRACT

Balaton is the biggest lake of Hungary (610 km^2) and one of the biggest in Middle Europe. Its shallow water renders it especially suitable for vacationists who are, however, attacked by swarms of mosquitoes that develop along the shore reeds and in pools, canals and swamps near the lake. Mosquito control in and around Lake Balaton, until 1978, was accomplished mostly by aerial treatments; from 1980 onwards only pyrethroids have been used. These chemical treatments killed not only mosquito populations but about 60-65% of the non-target arthropods as well. The discovery and development of *Bacillus thuringiensis* subsp. *israelensis (Bti)* enabled the selective control of mosquito larvae, without adversely affecting non-target organisms and the environment. The present paper reports experiments carried out in the Balaton area and presents important data on the efficacy and selectivity of *Bti.* Since 1987 an increasing area (from 200 to 2,000 hectares) has been treated around Lake Balaton with different *Bti* preparations (ABG-6108, Thurimos, Teknar, Skeetal) and a further increase of the area to be under integrated biological control is planned.

KEY WORDS: Lake Balaton, mosquitoes, microbial control, *Bacillus thuringiensis* subsp. *israelensis.*

INTRODUCTION

Lake Balaton, the largest standing water in Hungary and one of the biggest in Middle Europe, is situated in Western Hungary and is surrounded by volcanic cones and remnants of the Hungarian Central Mountain Range. This part was covered in the Tertiary Period by an inland sea (Pannonian Sea) that left many sediments, but the lake itself is younger and not of maritime origin.

The lake is about 77 km long (Fig. 1) and in most parts 5-7 km wide; at the tip of the Tihany Peninsula that divides the lake into two basins, the distance to the southeastern shore is only 1.5 km. The water is quite shallow (3^1 m deep) and only in the Tihany Strait it is 10-11 m deep. The surface of the lake is 610 km^2, with a volume of about 18 x 10^9 m^3 [the somewhat smaller but deeper Lake of Geneva (Lac Leman) contains in comparison 90 x 10" m^3]. The lake is fed at its southwestern end by the river Zala that carries 100-200 m^3/sec water; about the same

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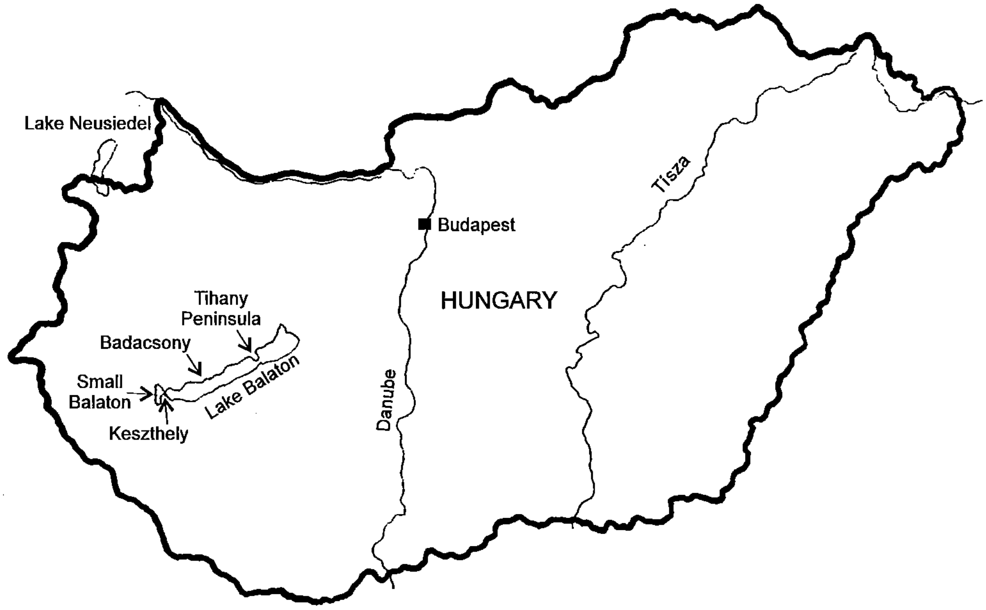

Fig. 1. Hungary and Lake Balaton.

amount comes from the canals of Héviz and Nagyberek and the smaller tributaries running into the lake. The excess water is drained into the Danube by the Sió canal, opened supposedly by the Romans who occupied Pannonia 2000 years ago. At the southwestern end of the lake (near Keszthely) there is a former swamp called Kis Balaton (Small Balaton) that was nearly completely drained and used as farmland, but is at present reconstituted to decrease the deposition of silt carried by the Zala river.

The oxygen content of Lake Balaton's water is high, the water itself slightly alkaline (pH = 8.6), turbid with colloidal $CaCO_3$ precipitated by assimilating algae. The water temperature in the period from June to September is mostly over 20°C; in winter the upper surface freezes but near the bottom water temperature remains around 4°C (Entz and Sebestyén, 1942).

The extremely colourful arthropod community around the Balaton contains also large populations of mosquitoes that develop partly in the lagoons and reed stands of the lake itself and partly in the smaller pools, canals and swamps of the surrounding area. Here especially the earlier studies of Makara and Mihályi (1943) Mihályi and Soós (1952), Mihályi (1955), Mihályi and Sz. Gulyás (1963) and, more recently Tóth (1991) have to be mentioned; thanks to their efforts we possess a clear picture of the composition of the mosquito fauna of both the Pannonian Region and the Balaton area itself.

According to Tóth (1991) 22 mosquito species molested vacationers during the 15-year period between 1973 and 1987. The five significant species were (Fig. 2): *Mansonia richiardii* Ficalbi (45%), *Aedes vexans* Meigen (29%), *Ae. annulipes* Meigen (13%), *Ae. sticticus* Meigen (4%) and *Culex modestus* Ficalbi (3%), adding up to 94% of the mosquitoes collected. The remaining 6% consisted of 17 species (*Ae. cinereus* Meigen; *Ae. excrucians* Walker; *Ae. rossicus* Dolbeskin, Gorickaja, Mitrofanova; *Anopheles bifurcatus* Linnaeus;

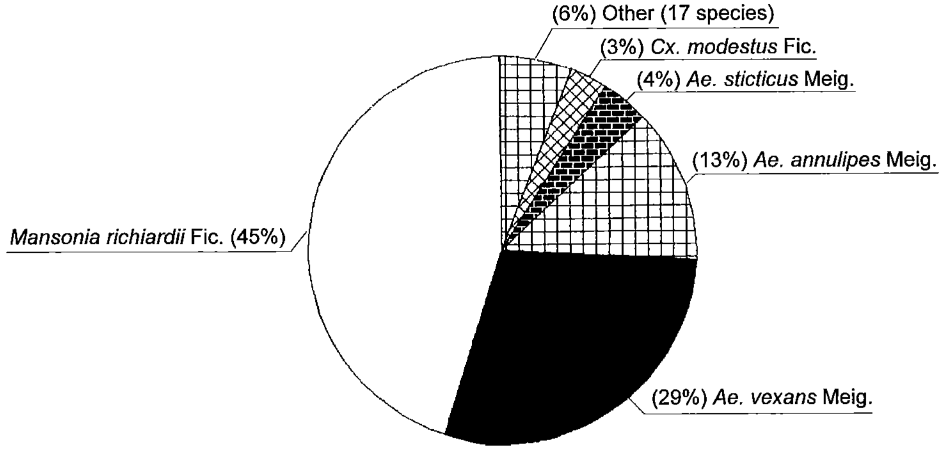

Fig. 2. Species distribution of attacking mosquitoes (45,000 individuals) collected at Lake Balaton in 15 years.

An. maculipennis Meigen, etc.). The species composition varied from year to year. In 1993, for example, *Ae. annulipes* Meigen was the most prevalent species, while *Ae. vexans* Meigen and *M. richiardii* Ficalbi were less prevalent, in comparison to other years. There are considerable differences in the individual density of *M. richiardii* between the northern and southern sides of the Balaton (Fig. 3). The lower densities of *Mansonia* are somewhat compensated for by the higher numbers of *Ae. vexans* and *Ae. annulipes*.

Unlike tropical and subtropical countries, mosquitoes do not present a health problem in Hungary (e.g. by transmitting pathogens of malaria, yellow fever or onchocerciasis); however their influence on social and public feeling is immense. A special Commission, established at Lake Balaton, to monitor the efficacy and secondary consequences of aerial chemical control, estimated the changes in the density of both mosquitoes and non-target organisms. The Commission established that about 150–200 non-target organisms were killed for every adult mosquito killed. Chemical control (using mostly pyrethroids like K-Othrin 1 ULV, Reslin Super 1 ULV) led to the annihilation of about 65% of the arthropods around Lake Balaton.

The discovery of *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) (Goldberg and Margalit, 1977; de Barjac, 1978; Margalit and Dean, 1985) and its consequent development and commercialization provided the means for selectively controlling mosquito larvae without affecting the non-target organism populations, including natural enemies. Reports of experiments carried out mostly in countries with warm climates (Ali, 1981; Ali et al., 1981; Dame et al., 1981; Davidson et al., 1981; Garcia et al., 1980, 1981; Ignoffo et al., 1981; Larget and de Barjac, 1981; Lacey et al., 1982; Purcell, 1981; Sebastien and Brust, 1981; Sinegre et al., 1979) and under different ecological conditions (brackish water, sewage canal, flood area, etc.), provided evidence for the high efficacy of *Bti* against mosquito larvae and its lack of activity against non-target organisms. The innocuity of the bacterium against these and warm-blooded organisms was verified earlier by many authors (de Barjac, 1981; Molloy and Jamnback, 1981; Siegel et al., 1987; Schnetter et al., 1981; Weiser and Vankova, 1978).

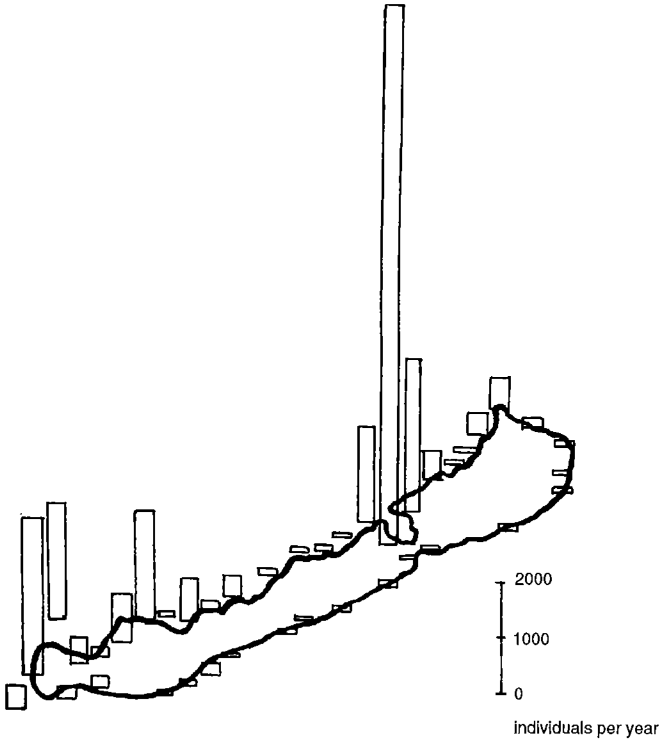

Fig. 3. Occurrence of *Mansonia richiardii* Fic. at Lake Balaton in 1986.

During the period between 1981 and 1984 we tested *Bti* in numerous laboratory and field experiments to determine: susceptibility of several mosquito species indigenous to Hungary; speed of action; dose-response curves; and effect on non-target arthropods (Copepoda, Cladocera, Amphipoda, Ephemeroptera, Heteroptera, Chironomidae, Dolichopodidae, Ephydridae) (Gharib and Szalay-Marzsó, 1982; Szalay-Marzsó and Gharib, 1982, 1983; Szalay-Marzsó, 1985, 1986, 1988).

The preparations ABG-6108 (Abbott, 100 IU), Thurimos (Sandoz, 1500 IU), Teknar (Sandoz, 1500 IU) and Skeetal FC (Novo Nordisk Industries) proved to be very effective against mosquito larvae while having no effect on the other aquatic organisms, enabling us to carry out large-scale field experiments.

In early August 1984 a field application of Teknar at a dosage of 1.5 kg/ha was made over the reed-covered shore of Lake Balaton using a helicopter (Corax Company) (Fig. 4). About 5 hectares were treated at a low altitude of ca. 4 m over a narrow, but navigable canal leading across the reeds into Lake Balaton. Efficacy was assayed using L_3 larvae of *Culex pipiens* placed in modified plastic containers, the sides of which were cut open and replaced by gauze, to allow free flow of water (interchange with the surrounding water). Twenty replicates with 50 larvae each were included in the experiment; 10 replicates were placed under reed foliage overhanging the canal and 10 under the open sky.

Fig. 4. Field application by helicopter of a *Bti* product over the reed-covered shore of Lake Balaton.

After 24 hours the following larval mortalities were established:

In containers under "open" sky	98.2 ± 0.7%
In containers partially covered by reed	75.1 ± 8.1%
Untreated	0.0

Another type of evaluation was carried out using swimming isolator tents (1 m^2 each) in order to study the mosquitoes and non-target organisms emerging from the submerged vegetation and mud. Insects were collected in glass traps that were emptied weekly for 3 weeks following the application.

No significant differences between the treated and untreated groups were found, with the exception of Culicidae that were represented by *An. maculipennis* and *Cx. modestus* (82.6 and 17.4% respectively) (Table 1). The larvae of the latter develop in the shallow water near the water edge and the adults feed mostly on anglers. There were no substantial changes in the number of chironomids (*Camptochironomus* and *Chironomus*) that would have indicated an adverse effect of the treatment on these non-target organisms. The group designated in Table 1 as Diptera div. contained flies that develop in the mud (Ephydridae, *Halophilus* spp., *Limonia tripunctata* Fabricius). The presence of the minute, beautiful, silver-sided *Uranotaenia unguiculata* Edwards (385 specimen) in treated water was unexpected, as this species was regarded as a rarity, represented only by a few exemplars in the Hungarian Museum of Natural History. Its appearance was surprising also inasfar as *Uranotaenia* is listed as a *Bti*-susceptible species (Goldberg and Margalit, 1977). Ephemeroptera were mostly represented by the common *Cloeon dipterum* Linnaeus; the Lepidoptera by *Cataclysta lemmata* Linnaeus, the larvae of

TABLE 1
Average number of faunal elements per isolator tent (1 m^2) in 3 weeks (Badacsony, 1984)

	Untreated	*Bti* treatment	$SD_{5\%}$
Diptera div.	34.333	44.333	33.333
Culicidae	14.667	1.167	12.212
Chironomidae	16.667	16.833	23.542
Uranotaenia	18.333	38.500	58.774
Limonia	2.833	4.333	6.455
Ephemeroptera	35.667	20.343	89.685
Hymenoptera	3.667	4.167	3.161
Lepidoptera	4.333	2.333	2.812

which live on aquatic plants; and Hymenoptera mostly by parasitoids (Chalcididae, Braconidae, Ichneumonidae) living inside reeds.

The field experiments carried out in 1986 and 1987 were aimed mostly against *Mansonia richiardii,* as stated above one of the most prevalent mosquitoes at Lake Balaton, the larvae of which live on aquatic plants and occur in the water from mid-summer until the following June.

The helicopter treatment was carried out on a warm (24°C) day of late September 1986 in an area of 30 hectares along the south-western shore (Keszthely). The experiment was continued in the following spring (28 May 1987) with the same dosage (1.5 kg Teknar/hectare) applied on 66 hectares, such that 10 hectares overlapped with those that had been treated in September. The entire experiment, therefore consisted of an untreated check; an autumn 1986 treatment; a spring 1987 treatment; and a section treated at both times. In each of the sections, 10–10 swimming isolator tents (1 m^2 each) were placed over rooted aquatic plants (reeds, bullrushes, sedges) that had been cut at water surface. The catches were then collected weekly for the following 8 weeks.

The summarized catches of 8 weeks are presented in Table 2. With the exception of Diptera there were no significant differences (at $P = 5\%$ level). Small differences could be attributed mostly to variation in biotopes of the large area used, as sometimes the lowest numbers

TABLE 2
Summarized number of faunal elements caught in 8 weeks (Keszthely, 1987)

		Bti treatments		
	Untreated	a: autumn 1986	b: spring 1987	$SD_{5\%}$
Chironomidae	2,073	2,578	2,500	147.150
Diptera	457	1,039	1,135	46.106
Ephemeroptera	724	206	431	46.521
Leptoceridae	145	129	176	9.968
Culex modestus	149	197	216	28.091
Hymenoptera	11	81	13	6.890
Odonata	11	2	11	1.472

occurred in the untreated check. The higher number of Chironomids (*Camptochironomus, Glytotendipes* and *Chironomus* species) was very reassuring but not unexpected, because this part of Lake Balaton shows the most intensive silting up.

The comparatively high number of *Culex modestus* individuals can be explained by the fact that the treatments did not reach the larval form at any time. It was, however, important that the adults were trapped exclusively during a period of two weeks (between 16 July and 2 August), so the knowledge of their swarming periods can be considered in future control operations.

A significant part of the Diptera belonged to Ephydridae (*Notophila riparia* Fallén, *Dichaeta caudata* Fallén, *Scatella stagnalis* Fallén), Dolichopodidae, Chloropidae or Cordyluridae; the family Stratiomyidae was represented by *Odontomyia hydroleon* Linnaeus.

A substantial part of the biomass was again accounted for by dayflies, practically by a single species: *Cloeon dipterum* Linnaeus. The caddisflies (Leptoceridae) were represented mostly by *Oecetis ochrana* Curtis. The dragonflies and damselflies were represented by *Agrion puella* Linnaeus, *A. pulchellum* Lindner and *Platycnemis pennipes* Pallas. The few Hymenoptera collected (Braconidae, Ichneumonidae) may have developed in insects mining in aquatic plants.

In retrospect our task of providing vital information for the integrated biological control of mosquitoes in and around Lake Balaton is solved by the multitude of data we have gathered on control of nuisance mosquitoes; we failed, however, to control *Mansonia richiardii* Ficalbi, the most prevalent nuisance mosquito that developed obviously in regions of permanent waters other than the ones treated at the lakeshore. To develop a control program based on the use of *Bti* and other environmentally safe methods we will have to survey and map the biotopes that produce annual swarms of mosquitoes to be able to treat them in a timely manner, as done in Germany along the Rhine River (Becker, 1990). Unfortunately effective mosquito control is not presently feasible without chemical means; however, we are striving toward a control program based on environmentally safe solutions. The usage trend of biological as opposed to chemical

TABLE 3
Mosquito control at Lake Balaton in the last 14 years

	Treated area (in thousand hectares)	
Year	Chemical (pyrethroids)	Biological (*Bti*)
1982	72	–
1983	90	–
1984	60	–
1985	60	–
1986	54	0.2
1987	50	0.2
1988	28.5	1.2
1989	16.5	2.0
1990	17.4	2.0
1991	16.9	2.0
1992	15.3	0.4
1993	10.7	1.4
1994	12.6	1.2
1995	6.8	1.6

products over the past 14 years is on the rise [as shown in Table 3, presented by S. Tóth at the 1994 Meeting of the Balaton Commission; the original table has been completed by the Corax Company with the last year's (1995) data]. I do not have to emphasize that the present management of Lake Balaton will do everything possible to increase the area treated with biological control agents.

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