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THE USE OF *BACILLUS THURINGIENSIS* SUBSP. *ISRAELENSIS (BTI)* AGAINST MOSQUITOES, WITH SPECIAL EMPHASIS ON THE ECOLOGICAL IMPACT

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ABSTRACT

For centuries man has taken measures to control mosquitoes in order to protect himself from mosquito borne diseases and annoyance. The control of mosquitoes by *Bacillus thuringiensis* subsp. *israelensis (Bti)* products offers man a means to serve several of his interests, viz. to protect himself from mosquitoes and to protect nature by taking account of the requirements of modern environmental protection. After careful screening, more than 200 tonnes of *Bti* are now used annually worldwide in campaigns for mosquito control, without evidence of any harmful impact on the environment. In Germany 97 cities and municipalities along a 310 kilometre stretch of the Upper Rhine River, with a total population of 2.5 million people, have joined forces to form the German Mosquito Control Association (GMCA/KABS), to control mosquitoes, mainly the flood-water mosquito *Aedes vexans,* over a breeding area of some 600 km^2 of the Rhine's flood-plain. The control of *Aedes* mosquitoes in Germany is based solely on the use of *Bti* products. The control strategy is elaborated on the basis of precise mapping of the breeding sites which takes not only the mosquito population but also ecological considerations into account. In the past years (1981-1996) some 37 tonnes of Sri powder or almost 1,000 tonnes of *Bti* granules as well as 29 tonnes of *Bti* liquid concentrates have been used in Germany, treating over 1,000 km^2 of breeding area, resulting in a reduction of the mosquito population by more than 90%. In an extensive monitoring programme the enviromental safety of *Bti* treatments is confirmed for each routine treatment. All investigations have shown that the numbers of *Aedes* mosquitoes are drastically reduced but that all other insects continue to develop in the water and provide, as winged adults, a food resource for birds, amphibians and bats.

KEY WORDS: *Bacillus thuringiensis* subsp. *israelensis,* mosquito control, ecological impact, Rhine River, German Mosquito Control Association.

INTRODUCTION

In temperate latitudes, the most troublesome species of mosquitoes [e.g. *Aedes vexans* (Meig.), *Ae. caspius* (Pall.), *Ae. detritus* (Hal.)] commonly breed in ecologically sensitive areas where broad-spectrum insecticides can not be used because of their side-effects.

The control of mosquitoes using *Bti* products offers man a means to serve several of his interests, viz. to protect himself from nuisance and vector mosquitoes and to protect nature by taking into consideration the requirements of modern environmental protection.

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Already a few years after its discovery *Bti* was being used on a large-scale for mosquito control in Germany. In addition to its environmental acceptability, *Bti* has a number of advantages that are responsible for the explosive development in its use, for example its relatively simple production on an industrial scale, the availability of specific and highly effective formulations, the improbability of resistance developing, the easiness of storage, and the simple mode of application.

After careful screening of other alternatives, since 1981 the control of mosquitoes in Germany has been based more or less solely on the use of *Bti*.

THE GERMAN MOSQUITO CONTROL ASSOCIATION (GMCA/KABS)

The control of mosquitoes in Germany has a long history. In the 1920s and 1930s breeding sites were treated with petroleum oils (Becker and Ludwig, 1983). During the 1950s and 1960s adulticides were used. In the early 1970s, there was no control. In 1975, the mosquito population was extremely high because of frequent fluctuations of the water level of the Rhine. The people in the villages could not spend any length of time outside their houses. There was an attack rate of more than 500 female mosquitoes/person/minute. As a reaction to this natural disaster 44 towns and communities on both sides of the Rhine River merged their interest in our united mosquito control programme, the GMCA/KABS, which was founded in 1976 under the leadership of Dr. Paul Schädler. Nowadays 97 cities and municipalities along a 310 kilometer stretch of the Upper Rhine River, with a total population of 2.5 million people, have joined forces to control the mosquitoes, mainly *Ae. vexans*, over a breeding area of some 600 km^2 of the Rhine's flood-plain. The budget of the program is approximately 3 million DM per year, which results in overall costs of approximately 1 DM per person per year (Becker and Margalit, 1993).

The overall goal of the GMCA/KABS is the integration of the needs of humans to be protected against mosquitoes and the protection of nature or rather the conservation of biodiversity. When we compare the ecosystem with a web and each group of organisms represents one mesh, the strategy of the GMCA/KABS aims at the removal of one single mesh representing the mosquitoes, without cutting other meshes in the "food web". This goal can be reached optimally only when biological control methods are being used.

The control of *Aedes* mosquitoes in Germany is based solely on the use of *Bti* products. Domestic mosquitoes [*Culex pipiens molestus* (L.)] are controlled mainly by the use of Culinex®-*Bti*-tablets in containers and septic tanks, as well as by the application of *Bacillus sphaericus* (*Bs*) to eutrophic ponds and ditches. Conservation and promotion of predators is an important goal of our programme. Therefore, microbial and biological methods are integrated with environmental management (e.g. improving the ditch system for regulation of the water level and providing permanent habitats for aquatic predators such as fish).

Prerequisites for the implementation of microbial control measures

The following are essential prerequisites for the implementation of microbial control agents in control programmes (Becker and Rettich 1994):

- **Entomological studies** concerning the biology and ecology of the native nuisance (vector) mosquito species; e.g. species composition and population dynamics related to climatical conditions;

- **Precise mapping** and numbering of all major breeding sites for quick reference during the operation;
- Assessment of the **minimum effective dosage** in bioassays with field collected larvae (LC_{99} = minimum effective dosage);
- Assessment of the **optimum effective dosage** in small field tests (2, 4, 8 or more times the minimum effective dosage) in dominating types of breeding sites under various abiotic and biotic conditions;
- Adaptation of the **application technique** to the requirements in the field;
- Design of the **control strategy** based on the results obtained during the preparation phase;
- Training of the **field staff** as the strongest asset of the field operation;
- **Governmental application formalities,** e.g. permission for the use of microbial control agents in protected areas.

Mapping of the breeding sites

The first step was to map all the breeding sites and to number them according to a special system. In this way each site can be located easily and the size of the breeding area calculated, once the routine application of *Bti* begins. Several parameters should be established during the mapping:

- Assessment of the **diversity and abundance of mosquito species** in the control area;
- **Characterization (typing) of the breeding sites** according to their productivity (densities) and population dynamics concerning mosquitoes;
- Assessment of the **ecological conditions** of the major breeding sites;
  - Assessment of the **plant associations** which indicate the degree of moisture (frequency of floods) of an area, based on the tolerance of plants against floods of a special area;
  - Assessment of the occurrence of **predators**;
  - Assessment of the occurrence of **rare and sensitive organisms.**

Assessment of the efficacy of various *Bti* products

Before any particular *Bti* formulation is used for a large-scale campaign, its efficacy against the most important mosquito species is tested in the laboratory and the optimal effective dosage is worked out during small-scale field trials.

In the preparation phase of our programme the efficacy of the products against the native mosquitoes was assessed in the following steps:

- Assessment of the activity of the products in bioassays after long-term storage;
- Assessment of the minimum effective dosage against native mosquito species in bioassays (LC_{99} = is defined as the minimum effective dosage);
- Assessment of the optimum effective dosage in small-scale field tests (e.g. 2, 4 and 8 times the LC_{99}).

As shown in Table 1, these tests revealed the optimum effective dosages/hectare for the *Bti* products mostly used.

The design of the control strategy

On the basis of the results achieved during the preparation and mapping phase the control strategy was worked out before embarking on the large-scale operation (Becker et al., 1992; Becker and Rettich, 1994).

TABLE 1
Dosages of various *Bti* products used in the routine programme against mosquitoes in Germany

	Optimum effective dosage/hectare *Aedes vexans* instars	
Product	L_1/L_2	L_3/L_4
Bactimos PP (10,000 ITU/mg)	125 g	250 g
Vectobac TP (5,000 ITU/mg)	250 g	500 g
Vectobac 12 AS (1,200 ITU/mg)	1 litre	2 litre
Vectobac G (200 ITU/mg)	7.5 kg	15 kg
Bti-sand granules (360 ITU/mg)	10 kg	20 kg

Amount in ITU: approximately 2.5×10^9 ITU/hectare.

The strategy is elaborated according to the following considerations:

- Adaptation of the control technique to the **ecological conditions**. According to ecological conditions, such as water-level and vegetative growth, the application of *Bti* may be made on foot or by helicopter. A helicopter is preferred, for example, when the site is difficult to walk through or when there are plants that may be crushed underfoot or animals that are sensitive to disturbance.
- Consideration of the **migration behaviour** of the target mosquitoes. The objective of the strategy is to keep mosquitoes out of human settlements, and thus also the migratory behaviour of the nuisance mosquitoes has to be considered. Species like *Ae. vexans* that migrate readily have to be controlled even in breeding sites that are far away from settlements (up to 15 km, which is the distance that *Ae. vexans* can migrate when population pressures are high). Snow-melt mosquitoes such as *Ae. cantans* (Meig.) do not migrate readily and have to be controlled only in a buffer zone of about 2 km around settlements. Domestic mosquitoes (*Cx. pipiens*) which migrate no more than a few hundred metres are destroyed only within the settlements and within a radius of 500 metres.
- The **potential productivity** of mosquitoes at a breeding site is a criterion for the relevance of a breeding site (assessment of the mosquito threshhold for the control). Whilst formulating the plan of campaign, the productivity of each breeding site must be taken into account. As a rule the larval density varies from site to site. It is not uncommon for sampling to reveal several hundred *Aedes* larvae per litre of water; sometimes, however, larvae occur only singly. Control measures against *Aedes* species are undertaken only in those sites where sampling regularly produces more than 3 larvae per litre.
- The **climatic conditions** (changes of the water level, rainy and dry season) influence the occurrence of the mosquitoes;
- The **population dynamics** of the target organisms determines the best timing of the treatment causing the strongest negative impact on the target organisms;
- The residual effect of the microbial control agent can be relevant for the **sequence of treatments**;
- Development of an **Integrated Control Strategy** (including predators, environmental management);
- Stimulating the **community participation**.

Routine treatments

As a rule the flood-plains of the Rhine are inundated 2 to 4 times each summer. The extent of the flood-water depends on the snow-melt in the Alps and on rainfall, and it is constantly necessary to monitor the water flow in the Rhine and in the flood-plain. During flooding *Aedes* larvae hatch within minutes or hours from the point of time of the temperature exceeding 10°C. Before control measures are initiated, the larval density and the larval stages are checked by means of 10 sample scoops at each representative breeding site. This is done in order to justify the action being undertaken and to establish the correct dosage and the best *Bti* formulation to be used. One day after application, spot samples are taken with a scoop at the reference breeding sites in order to check mosquito density and thereby establish the efficacy of the treatment.

According to the extent of the flooding, 10–20% of the potential breeding area of 600 km^2 has to be dealt with regularly by the 400 collaborators of the German Mosquito Control Association. During the worst floods, one-third of the area is treated with *Bti* granules dispensed by helicopter.

From 1981 until 1996 37 tonnes of *Bti* powder and almost 1,000 tonnes of *Bti* quartz sand granules as well as 29 tonnes of *Bti* liquid concentrates have been used to treat over 1,000 km^2 of breeding area.

Control of domestic mosquitoes is mainly carried out by the householders themselves. To assist in this, GMCA/KABS provides information on the biology of *Cx. pipiens molestus* and on appropriate control measures. Culinex® tablets have been exceptionally successful. They are based on *Bti*, and kill *Culex* larvae in water containers over a period of several weeks.

Monitoring the mosquitoes

In order to monitor mosquito abundance, a programme has been set up that involves 40 comparable sites throughout the entire drainage area. These are monitored twice a month from April to September, for a whole night on each occasion, and mosquito density is sampled by means of CO_2 light traps. Catches in areas where no control measures have been undertaken serve as points of reference (100% of the population) for catches from areas being controlled, in order to determine the success of the control measures (mortality rate in percent). It has been shown that since the widespread application of *Bti* began in 1981, more than 90% of the population of *Ae. vexans* has been killed each year and, despite extremely serious flooding in the last few years, mass occurrences of mosquitoes have been successfully averted. Naturally these control measures had an extraordinarily positive reception among the local people.

The close supervision of mosquito abundance in both flooded and built-up areas enables forecasts to be made of the migratory movements of the mosquitoes.

Monitoring the environmental impact

It has been essential to document the environmental impact of *Bti* application in order to provide a scientific basis for rebutting the arguments commonly advanced against mosquito control by its opponents. Before large-scale application of the *Bti* method was undertaken, the most important members of various aquatic groups (Cnidaria to Amphibia) had been screened in the laboratory and in small-scale field trials for their susceptibility to *Bti*. This work showed that in addition to mosquitoes and black flies only a few species of midges were affected by *Bti*. For the most part these midges were much less susceptible to *Bti* than the target organisms. For ten

years laboratory studies have been conducted to investigate the effect of the treatment on susceptible species such as the chironomid midges, and so far these have confirmed the bioassays on enviromental compatibility.

Monitoring the direct impact of *Bti* treatments

The development of insects in treated and untreated water bodies is continuously monitored using emergence traps (photo eclectors). The occurrence of insects in treated areas is assessed by regular light trap catches. All investigations have shown that the numbers of *Aedes* mosquitoes are drastically reduced but that all other insects continue to develop in the water and, as winged adults, provide a food resource for birds, amphibians and bats.

Monitoring the indirect impact of *Bti* treatments

The effect of a reduction in the number of mosquitoes on the food-chain was also studied. To this end, the food of birds (e.g. *Delichon urbica* and *Acrocephalus scirpaceus*), amphibia (e.g. *Rana* spp., *Bufo* spp. and *Hyla arborea*) and the niche utilisation and feeding preferences of bats (e.g. *Myotis daubentoni*), were determined. All investigations have shown that *Aedes* mosquitoes form no part, or only a very minor part, of the food-chain.

Resistance

Mosquito populations are checked at regular intervals for the development of resistance. No resistance has been detected after 10 years of treatment with *Bti* (Becker and Ludwig, 1993). To prevent resistance to *Bs* developing in *Culex*, *Bs* and *Bti* are used alternately in the control management plan for this species.

Altogether, some 8% of the GMCA resources are invested in these ancillary scientific investigations.

All the studies carried out to date have shown that the introduction of *Bti* has reduced the numbers of nuisance mosquitoes to a tolerable level, but that the diversity and beauty of the ecosystem as a whole have not been damaged.

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