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MICROBIAL CONTROL OF MOSQUITOES IN WEST GERMANY AND THE HUBEI PROVINCE OF THE PEOPLE'S REPUBLIC OF CHINA

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ABSTRACT

In West Germany, along major river systems such as the Upper Rhine, the nuisance caused by floodwater mosquitoes very often diminish the quality of life of the local residents. In the Province of Hubei in the People's Republic of China mosquitoes are vectors of malaria, Japanese-B-Encephalitis, and lymphatic filariasis. Due to the incurring resistance, and high development costs, as well as the environmental problems caused by common and newly developed insecticides, the control of these mosquitoes has become increasingly more difficult.

In both parts of the world, microbial agents such as *Bacillus thuringiensis israelensis* and *Bacillus sphaericus* have allowed an effective, cost efficient, and environmentally safe control of these mosquitoes. In West Germany the mosquito population has been reduced about 90% per year by applying approximately 5–10 tons of *B.t.i.*, depending on the fluctuation of the Rhine River water level, in about 500 km^2 of floodlands. In Hubei, each year about 12 000 hectars of rice fields and sewage ditches are treated with B.t.–187 (local strain) and *B.s.* C3–41 (local strain) preparations within an integrated control program against *Anopheles sinensis* and *Culex pipiens quinquefasciatus.* The incidence of malaria has thereby been reduced from 5.6 in 1986 to 0.8/10,000 people in 1989.

1. INTRODUCTION

Mosquitoes are worldwide the most common vectors of dangerous diseases such as malaria, arbovirus diseases, or filariasis. According to WHO estimates, more than one hundred million cases of malaria occur worldwide each year. More than 2.5 billion people live in areas where malaria is highly endemic.

For example in the Province of Hubei more than 20 million people on both sides of the Yangtsekiang River are threatened by the malaria agent *Plasmodium vivax.* During 1985 more than 50,000 cases of malaria were reported, which corresponds to a yearly incidence of 10/10,000 people. The major vector is the fever mosquito *Anopheles sinensis*, the control of which has recently become more and more difficult with the common insecticides, because of developing resistance as well as ecological and toxicological risks. Other diseases transmitted by mosquitoes are Japanese-B-Encephalitis, a virus disease transmitted by *Culex pipiens quinquefasciatus, Cx. tritaeniorhynchus, Aedes albopictus,* and *Anopheles sinensis,* and also Brugian and Bancroftian filariasis, transmitted mainly by *An. sinensis* and *Cx. pipiens quinquefasciatus.*

Owing to their massive occurrence along the larger river systems in Europe, mosquitoes, especially floodwater mosquitoes, can be a terrible nuisance. Due to the frequent fluctuations in the water level on the Upper Rhine Valley, mosquito populations there were often extremely dense. The dominant mosquito species are the floodwater mosquitoes *Aedes vexans, Ae. sticticus* and

Ae. rossicus, which breed in temporary water bodies resulting from rises in the water level of the Rhine River (Becker, 1989). Because of the nuisance caused by mosquito bites, the quality of life in the affected areas was often extremely reduced. During late afternoons and evenings, or on sultry days, the public was unable to spend any length of time outdoors. It was not uncommon for many hundreds of mosquitoes to attack a pedestrian in just a few minutes.

Culex pipiens molestus is the main pest in houses. Their breeding sites are usually rainwater containers near the houses and other water bodies occurring in summer.

2. CONVENTIONAL MOSQUITO CONTROL

For more than 40 years, chemical insecticides have allowed a relatively simple and economical control of almost all disease vectors. According to WHO calculations, in the early 1980's more than 50.000 t of chemical insecticides were applied yearly in those developing countries especially affected by vector-born diseases. Even today, DDT is the most widely used insecticide, representing approximately 50% of all insecticides used in the tropics. It was first believed that chemical insecticides alone could eradicate diseases such as malaria or onchocerciasis. However, many vectors soon became increasingly resistant to insecticides. New insecticides had to be developed at high costs, whereby the rapid development of new resistance could not be excluded. Besides the resistance problem, environmental problems such as accumulation in the food web and unselective effects, killing the predators of the mosquitoes as well, accompany the common insecticides. For these reasons the search for environmentally safe, biological methods of insect control was begun. Among all of the microbial control agents for mosquitoes and black flies, the spore-forming bacteria such as *Bacillus thuringiensis israelensis* and *Bacillus sphaericus* are the most promising. This shall be illustrated by two samples.

3. MICROBIAL CONTROL OF MOSQUITOES IN GERMANY

As a reaction to the nuisance caused by mosquitoes, approximately 100 towns and villages on both sides of the Rhine River joined together to form a voluntary mosquito control organization (KABS). The territory of this organization now covers approximately 300 river kilometers (Becker and Ludwig, 1983).

The objective of this program is the reduction of the mosquito population to a tolerable level without damaging the ecologically sensitive riverbank areas and their biocoenosis. This objective has been achieved by the widespread use of microbial agents.

One of the strongest assets of the organization is the well-trained local field staff. There are usually about 300 people active and the yearly budget is about 1 mill. U.S. dollars. Depending on the size of the breeding sites, in every community there are between two and eight people responsible for mosquito control. Besides the community workers, biology students familiar with the local area are employed during flood periods as temporary workers in the control of *Aedes* species, receiving a loan of 6 U.S. dollars.

On the one hand, an integrated control, applying different but compatible methods, reduces the development of resistance. On the other hand, it allows a more pest-specific control. It is important that, from the available methods, always those methods are chosen which are not only effective in the given situation but are also environmentally safe.

The following methods are applied in the mosquito control program in West Germany:

a) Microbiological Methods: For the last eight years, *B.t.i.*-preparations have been mostly used in routine control measures. Today the mosquitoes in more than 90% of the area exclusively are controlled by the KABS with *B.t.i.*. *Bacillus sphaericus* preparations are increasingly being used against *Cx. pipiens molestus*.

b) Water Management and Related Measures: The protection and encouragement of all natural predators is a very important maxim of our integrated methods. As the predators are not affected by microbial agents, they can continue to feed upon newly hatching mosquito larvae after the breeding sites have been treated. This also has economical consequences, since in most cases no further treatments are then necessary.

c) Surface film method: Only when *B.t.i.* is no longer effective, is a self-spreading biodegradable surface film, which is a mixture of soybean, lecithin, and paraffin applied in concentrations of 0.4–0.8 ml/m2 against late fourth instars and pupae.

In 1979 we started testing various available commercial *B.t.i.* products, such as BACTIMOS, TEKNAR, and VECTOBAC (Tables 1 and 2).

As a result of these tests, in routine treatments against first and second instar larvae or in shallow breeding sites, 250 g of *B.t.i.* powder (activity: 6000 AAU/mg) is mixed with 10 liters of screen-filtered pond water per hectar. The mixture is applied by field workers with a hand-held, high-pressure sprayer. On the other hand, in deeper breeding sites or when third or fourth instar larvae are present, 500 g of *B.t.i.* WP is used. This corresponds to about 1.8×10^9 AA International Toxic Units per hectar when about 300 g WP are applied.

In order to achieve a sufficiently high mortality rate under field conditions, one liter of fluid concentrate (Teknar HP-D) or Vectobac 12 AS) is mixed with 9 liters of screen-filtered pond water per hectar.

When high water levels on the Rhine cause widespread inundation or when dense vegetation occurs, *B.t.i.* granules formulated by ourselves are applied. This formulation consists of 50 kg of *B.t.i.* wettable powder (activity:6000 AAU/mg) in a cement mixer. This is enough to treat about 2 hectars of breeding site either by helicopter, equipped with a SIMPLEX sprayer, or by hand.

Between 1981 and 1989, more than 40,000 hectars of mosquito breeding sites were successfully treated with about 18 tons of *B.t.i.* wettable powder and 17,000 liters of *B.t.i.* flowable concentrate. The *B.t.i.* WP was also used to produce approximately 300 tons of *B.t.i.* sand granules.

The control of *Culex pipiens molestus* near houses is based on providing information to the general public on the biology of the house mosquitoes and strategies for their control. People are asked to destroy all unnecessary water bodies near their homes, to empty the rain water containers at least once a week, or to cover them thoroughly. Fish can also be used as predators or *B.t.i.* briquets can be applied in rainwater containers, the latter allowing efficient control for several weeks. For the last 5 years, we provided about 300,000 *B.t.i.* briquets to the public. Since 1989 we have been testing briquets on the basis of *Bacillus sphaericus* to optimize the *Culex pipiens* control.

As a result of all these measures, the mosquito population in the Upper Rhine Valley was reduced each year by over 90%. This has been highly respected by the public, especially regarding the low costs and the tremendous increase in the quality of life.

4. MICROBIAL CONTROL OF MOSQUITOES IN HUBEI PROVINCE

In a cooperative program between the Province of Hubei and Baden-Württemberg (West Germany), the scientific and organizational prerequisites for the large-scale application of microbial agents are being improved. In detail, the aims of this program are:

a) Intensive exchange of practical and scientific knowledge as well as technology in the field of integrated mosquito control between West Germany and the Province of Hubei.

b) Detailed investigations on the biology and ecology of the most important mosquito vectors of human diseases, as a basis for their successful control by microbiological means.

c) Development of low-cost and environmentally safe control measures, which can be applied at the community level.

d) Effective improvement of the measures and techniques for routine control operations in both countries in a long-lasting cooperation between the two working groups.

TABLE 1
Field evaluation of various wettable powder formulations of *Bacillus thuringiensis H-14* against larvae of *Aedes vexans* and *Aedes cantans*

Product	Treatment rate	Habitat surface/depth	Species instars	No. of larvae/10 dips pre- and post-treatment		
				Pretreatment	1	2 (days)
BACTIMOS WP (6000 AAU/mg)	0.1 kg/ha	Swampy woodlands $19.5m^2/0.15m$	*Ae. cantans* (L_3/L_4)	109	22 (79.8)	0 (100)
TEKNAR WP (5800 AAU/mg)	0.1 kg/ha	Swampy woodlands $18.2m^2/0.15m$	*Ae. cantans* (L_3/L_4)	57	43 (24.6)	5 (91.2)
VECTOBAC WP (3600 ITU/mg)	0.1 kg/ha	Swampy woodlands $16m^2/0.1m$	*Ae. cantans* (L_3/L_4)	131	119 (9.2)	67 (48.9)
BACTIMOS WP	0.2 kg/ha	Swampy woodlands $18m^2/0.1m$	*Ae. cantans* (L_3/L_4)	62	3 (95.2)	0 (100)
TEKNAR	0.2 kg/ha	Swampy woodlands $19.5m^2/0.15m$	*Ae. cantans* (L_3/L_4)	81	38 (53.1)	3 (96.3)
VECTOBAC	0.2 kg/ha	Swampy woodlands $24m^2/0.15m$	*Ae. cantans* (L_3_L_4)	106	76 (28.3)	1 (99.1)
TEKNAR WP	0.1 kg/ha	Floodlands $120m^2/0.2m$	*Ae. vexans* (L_2–L_4)	130	95 (26.9)	34 (73.9)
TEKNAR WP	0.2 kg/ha	Floodlands $120m^2/0.2m$	*Ae. vexans* (L_1–L_4)	260	2 (99.2)	0 (100)
BACTIMOS WP	0.2 kg/ha	Floodlands $80/m^2/0.2m$	*Ae. vexans* (L_1–L_3)	759	14 (98.2–	2 (99.7)

( ) = percent reduction; L_1–L_3 = larval instars

TABLE 2
Field evaluation of various flowable concentrates of *Bacillus thuringiensis H-14* against larvae of *Aedes vexans* and *Aedes cantans*

				No. of larvae/10 dips pre- and post-treatment				
Product	Treatment rate	Habitat surface/depth	Species instars	Pretreatment	1	2	3	4 (days)
TEKNAR 402 SC (1500 AAU/mg)	1 l/ha	Floodlands $225m^2/0.2m$	*Ae. vexans* (L_1–L_4)	386	7 (98.2)	1 (99.7)	0 (100)	0 (100)
TEKNAR 402 SC (1500 AAU/mg)	1 l/ha	Swampy woodlands $6.5m^2/0.2m$	*Ae. cantans* (L_2–L_3)	100	62 (38)	31 (69)	8 (92)	0 (100)
TEKNAR HP-D (3000 AAU/mg)	1 l/ha	Swampy woodlands $6.5m^2/0.2m$	*Ae. cantans* (L_2–L_3)	186	2 (98.9)	0 (100)	0 (100)	0 (100)
TEKNAR HP-D (3000 AAU/mg)	1 l/ha	Swampy woodlands $12m^2/0.3m$	*Ae. cantans* (L_3)	312	53 (83.1)	16 (94.9)	6 (98.1)	1 (99.7)
VECTOBAC-AS (600 ITU/mg)	1 l/ha	Swampy woodlands $11.2m^2/0.3m$	*Ae. cantans* (L_3)	328	241 (26.5)	51 (84.5)	44 (86.4)	5 (98.5)

( ) = percent reduction; L_1–L_4 = larval instars

4.1. Laboratory tests with Bacillus thuringiensis and B.s. preparations

During the first phase of the program, two *B.t.i.* powder formulations and four *B.s.* preparations were tested in the laboratory of the Institute of Parasitic Diseases in Wuhan (Hubei). The tests were carried out against larvae of *Cx. pipiens quinquefasciatus*, *An. sinensis*, and *Ae. albopictus* at concentrations of 0.001; 0.01; 0.1; 1 and 10 ppm. While the *B.t.i.* preparations were very effective against larvae of *Ae. albopictus* (LC50 = 0.025–0.0568 ppm), they were less active against *Cx. quinquefasciatus* larvae (LC50 = 0.037–0.28 ppm).

On the other hand, a relatively low dosage of *Bacillus sphaericus* was already enough to kill *Culex* larvae (LC50 = 0.001–0.009 ppm), whereas *Aedes* larvae are less sensitive (LC50 = 0.54–0.72 ppm). However, both microbial preparations did not provide satisfactory control of larvae of *An. sinensis* (LC50 = 1.9–10 ppm).

4.2. Field tests with B.t.i. and B.s.

The most effective laboratory preparations were tested against *Cx. pipiens quinquefasciatus* and *An. sinensis* in eight drainage canals and ponds.

The large scale field tests against *Cx. pipiens quinquefasciatus* larvae with the most effective preparations yielded highly sufficient results at concentrations of 0.5–1 ppm for about one week.

Besides testing for efficiency, we could also show that microbial agents have no negative impact to the ecosystem, especially to the predators of mosquitoes. These field tests, as well as the laboratory experiments, demonstrated that for the control of *Anopheles* larvae, more effective microbial preparations need to be developed in order to reduce the excessive control costs caused by high dosages and repeated treatments. In this context it was of interest to develop a slow releasing floating formulation for the control of *Anopheles* larvae as a "surface feeder."

During the last years, about 10 tons of *B.t.*-187 (local strain) and about 14 tons of *B.s.* C3–41 (local strain) have been produced each year by utilization of natural resources in Hubei Province, which was enough to treat about 12,000 hectars of mosquito breeding sites. In 1989 about 200 kg of new floating granules were used against *Anopheles sinensis* larvae treating an area of about 300 hectars of rice fields.

As a result of these measures the incidence of malaria decreased from 5.6 cases/10,000 people in 1986 to 0.8 cases/10,000 people in 1989 (Xu, Becker and Xianqi, 1990).

B.t.i. and *B.s.* preparations offer not only high efficiency against mosquitoes coupled with low treatment costs, but also complete safety for all non-target organisms, which can be promising agents in the fight against dangerous diseases such as malaria and filariasis. It can be assumed that, through applying these microbial agents in an integrated program, at least for an acceptable time-period, the onset of resistance can be prevented.

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