Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

CONTROL OF FLOODWATER MOSQUITOES IN WETLANDS

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Abstract Flood water mosquitoes have been successfully controlled in Switzerland for 25 years with products based on *Bacillus thuringiensis israelensis* (Bti). These mosquitoes have a flying range of up to 10 km in search of a blood meal. Before the interventions the quality of life of residents in villages near the mosquito breeding areas was greatly reduced, and tourism suffered financial losses. The logistic to block the mass development of the flood water mosquitoes, dominated by *Aedes vexans* and *Ochlerotatus sticticus* is demanding. Accurate forecast of precipitation in the breeding areas is an important for the planning stage. Flooded breeding sites require monitoring for the presence of mosquito larvae. Only a few days are available for Bti-interventions. Time slots for aerial treatments by helicopter have to be obtained. Ground application equipment has to be ready. Mosquito larvae have to be eliminated in their third instar, once in the fourth instar the efficacy of Bti drops. Post-treatment monitoring has to show 95% to 99% elimination of mosquito larvae. It is the goal to limit the Bti-treatments to one or two interventions per year. Bti-treatments to control flood water mosquitoes requires governmental approval.
Key words Mosquito control in wetlands, *Bacillus thuringiensis israelensis*.

INTRODUCTION

In temperate climatic zones, especially in Europe, mosquitoes were during the second half of the past century no major issue. Malaria had been eradicated, and other mosquito borne infectious diseases were mainly limited to the tropical belts. At present this situation is changing drastically, as will be highlighted later-on during this conference. Urbanization, human mobility, world-wide passive transport of mosquitoes as well as re-naturation and extension of wetlands is leading to an increased exposure of the European population to mosquitoes. This development is accelerated by the climate change, especially warming and extreme weather situations.
The impact by flood water mosquitoes which is emphasized here, on humans but also on farm animals seems to be on a steady increase due some of the above mentioned factors. In many instances control of these mosquitoes has become indispensable. The main breeding sites are located in wetlands which are periodically flooded. Wetlands are fragile ecosystems and need protection. Therefore, interventions with biocides is very delicate. The only approved biocides are based on *Bacillus thuringiensis israelensis*. The simultaneous work with two organisms, on the one hand side a bacterium on the other hand an insect, is highly demanding. The control of mosquitoes has to be effective, and at the same time the biosafety of the microbial control agent has to be assured. Thus the risk-benefit factor becomes a key issue along with the cost-benefit ratio.
The goal of our work was and is the control the mass development of flood water mosquitoes to limit the disturbance for residents in the vicinity of breeding sites. The flood water mosquitoes belong to the two genera *Aedes* and *Ochlerotatus*. *Aedes vexans* is the dominating flood water species in many European

countries including Switzerland. Their mass development is directly correlated with the severity of flooding (Rettich et al., 2012). The dynamic of these mosquitoes differs greatly from other mosquitoes. The eggs are deposited in moist soil, temporarily flooded usually during spring and early summer. Hatching occurs synchronously as soon as the eggs are inundated. Depending on the water temperature the development of the aquatic stages may take one to two weeks. The striking characteristic is the extreme high population densities with often more than 1’000 larvae per liter. Masses of adult females may appear after sunset in the search for blood meals, covering distances of up to 10 km. The longevity averages four to six weeks. A single blood meal is sufficient for the development of at least 100 eggs which are deposited again in temporarily flooded soil.
The only measure to curb the mass invasion of flood water mosquitoes into residential areas is the control of the larvae in their breeding places. The check the widely dispersed adults is not feasible. The use of adulticides in residential areas should be avoided. Insecticides such as pyrethroids provide only short-term relieve, and they are known for their potential side effects towards other insects such as bees.

MATERIALS AND METHODS

***Bacillus thuringiensis israelensis* (Bti).** Over the past twenty-five years the commercial product Vectobac-G® (Valent BioSciences, Libertyville, IL, USA) has been used exclusively for the control of the flood water mosquitoes. It is a granular formulation with potency of 200 International Toxic Units/mg. The product is based on a Bti strain designated as AM65-52. The toxic effect against the mosquito larvae is caused by the Cry-proteins Cry4, Cry10, Cry 11 supported by the Cyt-proteins, Cyt1 and Cyt2. All these proteins are coded by genes located on a mega plasmid designated as pBtoxis (Schnepf et al., 1998).
Application technique and equipment. The mosquito breeding areas in the Plain of Magadino bordering Lago Maggiore, and the upper end of the Lac de la Gruyére are treated from the air. Fertiliser spreaders with a loading capacity of 200 kg of Vectobac-G are hooked on to a helicopter. The Bti-granules are distributed by a rotating disk covering a swath of 20 m. The delivery rate of Vectobac-G is adjusted to 14 kg/ha with a flying speed of 60 km/h at an altitude of 80 m. In the smaller flooded sites of the natural reserve of the Thurauen where the river Thur joins the Rhine, treatments are carried out by ground equipment with a motorized knap sack sprayer adjusted for the delivery of Vectobac-G granules. The same application rates are used.
Pre-and post-treatment logistics. During spring and early summer months preparation for the interventions against the mosquitoes have to start on time. Weather reports and predictions of flooding, issued by the Federal Office for the Environment (FOEN) are used as early warning systems. The increase of the water levels in the project areas is followed via the internet. The field work starts with the monitoring of the mosquito larvae. If an intervention is necessary a slot for the helicopter treatment has to be requested. At the time of the treatment enough Vectobac-G, packaged in bags of 18.1 kg, has to be on the site. Including reloading, a helicopter treatment takes two to four hours depending on the extension of the flooding. Post-treatment controls for surviving larvae are carried out 24 h following the intervention. If required, the presence and density of adults is monitored with CDC traps.
Administrative issues. Each mosquito control project is supervised by a working group composed of representatives of the local population, of the Cantons and the Federal Government. Detailed reports on the Bti-interventions have to be submitted annually. Permission for the following year has to be granted by the various government offices.

RESULTS AND DISCUSSION

Efficacy of the Bti Treatments

The long-term interventions against the flood water mosquitoes carried out since a quarter of a century in the Plain of Magadino, and since 18 years at the Lac de la Gruyère were highly satisfactory. In 2013 a first Bti-treatment was performed in breeding sites near the village of Ellikon neighboring the Thurauen. The residents could be protected from major disturbances by mosquitoes which prior to the Bti-treatments reduced severely the quality of life during the summer months.

Decision Making Parameters for Interventions

The essential reference point is the degree of nuisance caused by the flood water mosquitoes to man. Thus, recurrent complaints by residents are the key factors. Complaints have to be verified and carefully recorded and inter-linked with the locations and size of the temporarily flooded zones and the concentration of the mosquito larvae. This information forms the basis for decisions to carry out Bti-treatments. Despite regular interventions the density of the mosquito larvae remained rather constant in a given breeding zone with numbers ranging between 100 and 1'000 per liter. The minimum number of larvae requiring an intervention is linked to the level of complaints and falls into a range between 3 and 30 larvae/liter. For example in the German mosquito control program along the upper Rhine valley the threshold is reached with 3 to 5 larvae/liter (n. Becker, personal communication) and for example with 30 larvae/liter in a mosquito abatement program in the Fraser Valley Regional District, Canada (2014).

Timing of Bti Treatments

The timing of Bti-operations is crucial and highly demanding. Synchronous hatching takes place as soon as the eggs are inundated. The minimum temperature is 10^0C. But 20^0C to 25^0C during spring and summer flooding are reached rapidly, shortening the development of the aquatic mosquito stages to 7 – 10 days. The efficacy of Bti is highest when applied to larvae in their 2^{nd} and 3^{rd} stages. The time window for the intervention is 2 to 3 days. The treatments should be carried at the last moment anticipating a possible decrease of the water level and the size of the flooded area. Once mosquito larvae have entered the 4^{th} and final stage, treatments cannot be postponed even if the weather forecast calls for more rain with an extension of flooding. In such situations another treatment is required, but covering only sites additionally covered by water with newly hatched larvae. The number of treatments per season has to be kept to a minimum if ever possible limited to one or two interventions.

Bti Application Rates

Long-term experience shows that under the conditions encountered in Switzerland a minimum dosage of 14 kg/ha of Vectobac-G is required to achieve a satisfactory reduction of the mosquitoes by 95% to 99%. This corresponds to about $3x10^9$ International Toxic Units/ha. The flooded wetlands to be treated are covered often with more than one canopy of dense vegetation consisting of trees, shrubberies, reed and grass. Hence a uniform distribution of about140 Vectobac-G granules/m^2 is required to reach the mosquito larvae in the water. Post-treatment controls at 24 h and 48 h will reveal the success of a Bti-treatment. After one day 90% of the larvae should be eliminated, and at day 2 surviving larvae should be encountered rarely. A re-treatment of the same flooded area due to the application of an insufficient Bti-concentration has to be avoided. The costs are too high and the addition of another dose of the biocide is not desirable.

The Cost-Benefit Relationship

Long-term control of flood water mosquitoes is only feasible if the benefit for the society is substantially higher than the costs incurred. Maintaining an acceptable quality of life for the residents within the perimeter of flood water mosquitoes cannot be measured in money. The key economic factors refer to losses incurred by restaurants and tourisms as well as costs for repellents providing at least partial protection from mosquito bites. The cost-benefit factor is the highest in the densely populated plain of Magadino with tourism being an essential source of income for the region. Invaded by mosquitoes, restaurants are not able to serve their guests in the open especially after sunset. In the plain of Magadino the cost-benefit factor was calculated at 1:50. The cost-benefit factor in the mosquito control project at the Lac de la Gruyère is about 1:5. Here the population affected amounts to 5‘000 persons being about ten times lower than in the Plain of Magadino, and the number of tourists spending their holiday is less. Within the third and smallest mosquito control project, initiated 2013 in the region of the nature reserve of the Thurauen the cost-benefit factor is at least 1:2, still in favor of the benefit. Ten ha were treated by ground application to protect the small village of Ellikon with 90 inhabitants and two restaurants visited by day tourists.

Biosafety of Mosquito Control with *Bacillus thuringiensis israelenis*

Bacillus thuringiensis in general, and in this context the subspecies *israelensis* has an outstanding biosafety record. Bti-products are used world-wide since decades in various formulations, adapted to the type mosquito breeding site. The safety is based on the unique properties of the Cry-proteins which differ among Bt-subspecies (Schnepf et al., 1998). The high specificity is due to a multistep cascade starting with the dissolution of the Bti-crystals via an enzymatic activation process leading to receptor mediated binding with lethal pore formation in the gut epithelium membrane of the target larvae.

The insecticidal activity of the Bti Cry-proteins supported by the Cyt-proteins is limited to a few genera of *Nematocera* (Diptera). Mosquito (*Culicidae*) species are the most susceptible. Many studies have demonstrated the safety of the Bti insecticidal proteins (Lacey and Siegel, 2000; Tetreau, 2012). The World Health Organization (WHO, 1999) approved the global use of Bti, recommending it as an addition to stored drinking water to prevent development of container breeding mosquitoes.

Bti-spores are applied along with the Cry-proteins. The long-term fate of the spores in the environment has been investigated by Guidi et al., (2011). Proliferation was not found. The spore concentration decreased continuously after each treatment.

Indirect impacts on the food web in areas where Bti is regularly applied has become a controversial issue. Poulin et al. (2010) found a reduction of the progeny of house martins caused by a decrease of Nematoceran prey species. Becker et al. (2010) did not find any evidence that mosquitoes served as a major nutrient source for birds. Very likely both authorships were correct. A temporary mass development of flood water mosquitoes could have led via the food web to an increased availability of nutrients for the house martins. Non-flood water mosquitoes are only marginally or not at all affected by the Bti-treatments since their life cycles vary, and they occupy different breeding sites.

Legal Aspects

The majority of wetlands as the main breeding sites of flood water mosquitoes are protected areas where intervention is generally banned. A control of flood water mosquitoes is only admissible if the disturbance caused in urban areas outweighs nature conservation. Products based on Bti are the only permitted biocides. Authorities approve the use of Bti in protected wetlands with great diligence and reserve. Maintenance of a balance between the different interests of the stakeholders remains a complex problem.

At present it is uncertain whether Vectobac-G may be used beyond 2014 since the approval granted by the Swiss authorities has expired on 30 September 2013 and the manufacturer has to our knowledge not filed a request for re-registration. The Bti-products held on stock may be applied for one more year. Switzerland follows closely the EU directives on biocides (The EU Biocidal Products Regulations No 528/2012).

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