PS4 PILOT STUDY

2014

MISTRA EviEM

MISTRA COUNCIL FOR EVIDENCE-BASED ENVIRONMENTAL MANAGEMENT

Biological control of mosquitoes using *Bacillus thuringiensis israelensis*: a pilot study of effects on target organisms, non-target organisms and humans

Magnus Land
Matilda Miljand

Mistra EviEM PS4 Pilot Study

Publication date: 09 April 2014

Please cite as: Land, M., Miljand, M., (2014): **Biological control of mosquitoes using *Bacillus thuringiensis israelensis*: a pilot study of effects on target organisms, non-target organisms and humans.** Mistra EviEM Pilot Study PS4 (www.eviem.se)

Biological control of mosquitoes using *Bacillus thuringiensis israelensis*: a pilot study of effects on target organisms, non-target organisms and humans

A Pilot Study

Magnus Land
Mistra EviEM
Royal Swedish Academy of Sciences
Box 50005, SE - 104 05 Stockholm, Sweden
Email: magnus.land@eviem.se

Matilda Miljand
Mistra EviEM
Royal Swedish Academy of Sciences
Box 50005, SE - 104 05 Stockholm, Sweden
Email: matilda.miljand@eviem.se

Abstract

The Swedish Environmental Protection Agency has been commissioned by the Swedish government to evaluate various effects of using VectoBac G to control the abundance of mosquitoes in flooded areas. VectoBac G is a granular formulation of the bacterium Bacillus thuringiensis subsp. israelensis (Bti). The efficacy of Bti treatment on target organisms has been thoroughly studied and shown to be very high. Direct effects on non-target species have been studied quite extensively, and the largest non-target group susceptible to Bti is chironomids. Due to its mode of action, Bti is considered to be safe for fish, birds and mammals, including livestock and pets. Despite a fairly large number of studies, no occurrence of resistance to Bti in the field has been reported. Effects on eutrophication of water bodies and indirect effects on non-target species related to changes in the food web have rarely been studied. Nuisance-related effects of the presence of mosquitoes on human well-being, recreational activities, tourism and home prices are poorly studied too. There are a fairly large number of studies on the effectiveness of Bti treatment in terms of mosquito abundance, although the results have varied. It may thus be feasible to conduct a systematic review in that area. The same is probably true also for the persistence of Bti in the environment.

Background

Several species of mosquitoes are known to be important vectors of diseases (Boisvert, 2007). Mosquitoes may also be a nuisance to humans and animals. Consequently, many attempts to control the abundance of mosquitoes have been made using various methods. In 1976, the bacterium Bacillus thuringiensis subsp. israelensis (*Bti*) was isolated and found to be toxic to mosquito larvae (Goldberg and Margalit, 1977), and since the early 1980s, *Bti*-based insecticides have been available commercially (Boisvert, 2007). In 2011, Bacillus thuringiensis subsp. israelensis Serotype H-14, Strain AM65-52, was included in Annex I to EU directive 98/8/EC concerning the placing of biocidal products on the market.

Bti is generally considered to be an effective and target-specific insecticide. The specificity has been explained by the mode of action of *Bti*, which generally is accepted to be a multistage process (Ramírez-Lepe and Ramírez-Suero, 2012). The toxic properties of *Bti* originate in crystalline inclusions produced during *Bti* sporulation. The crystals and their subunits are inert protoxins and are not biologically active. However, they become activated when ingested by mosquito larvae, and the mode of action can be outlined as follows: (i) ingestion of Cry protein by the larvae; (ii) solubilisation of the crystals in the alkaline (pH>10) midgut; (iii) proteolytic activation of the insecticidal solubilised protein; (iv) toxin binds to receptors located on midgut cell membranes; (v) a change in the toxin conformation allowing toxin insertion into the membrane; (vi) electrophysiological and biochemical evidence suggest that the toxins generate pores in the cell membrane, thus disturbing the osmotic balance and causing the cells to swell and lyse; (vii) the gut becomes paralysed and the insect stops feeding. Most mosquito larvae die within a few hours of ingestion. *Bti* has no effect on mosquito eggs, pupae or adults.

Bti-based products have been used in more than 25 countries on all continents (Boisvert, 2007). In Sweden, large-scale use of *Bti* has mainly occurred in wetland areas in the lower regions of River Dalälven, where application first started in 2002. From the start of the mosquito control programme, the Swedish Environmental Protection Agency (EPA) has financed an evaluation programme, and after seven years of operation (2002–2008), the evaluation programme was reviewed by Nilsson and Renöfält (2009). While critical in several respects, they emphasised a range of problems related to the scientific methods used (e.g. low statistical power) and the fact that the evaluation programme was conducted by the same people who conducted the mosquito control programme.

The use of *Bti*-based insecticides in Sweden is not entirely uncontroversial. One reason is that extreme mass occurrences of mosquitoes may in fact be induced by human activities (e.g. river regulations and eutrophication of surface water), and that it might be better to take action against those activities, rather than mosquitoes (Nilsson and Renöfält, 2009). Another reason is that treated areas in the lower River Dalälven basin are located close to, and even within, protected Natura 2000 areas. Questions have been raised as to whether *Bti* has negative effects on the environment and ecosystems in those areas (Karlsson and Terstad, 2012).

Identification of topic and stakeholders

The topic was suggested by the Swedish EPA, which has been commissioned by the Swedish government to evaluate various effects of using VectoBac G to control the abundance of mosquitoes in flooded areas. VectoBac G, GS (formerly CG) and GR are granular formulations of Bacillus thuringiensis subsp. israelensis Serotype H-14 (strain AM65-52) for control of mosquito larvae. The Swedish EPA will deliver a progress report to the Ministry of the Environment on 1 March 2014, and a final report will be delivered no later than 1 November 2015.

The Swedish EPA report will encompass effects of Vectobac G on target organisms, biodiversity (including protected environments and species), local residents, livestock and pets, recreational activities and tourism. The report will also address potential risks and consequences of development of resistance against the insecticide and eutrophication of the treated areas. Moreover, the Swedish EPA has been asked to report on possible cumulative effects of repeated treatments during several years. A systematic review of a topic within this field could serve as a background document for the final Swedish EPA report to the government.

Other stakeholders that the Swedish EPA has been mandated to consult in their work on this issue include the County Administrative Board in Gävleborg, the National Board of Health and Welfare, the Swedish Board of Agriculture, the National Veterinary Institute, the Swedish Forestry Agency, the Swedish Agency for Marine and Water Management, and the Swedish Chemicals Agency. The Swedish Chemicals Agency has authorized the use of VectoBac G under certain circumstances. However, if the product is to be released from airborne devices, or if protected areas will be affected, the Swedish EPA or the county administrative board will have to grant an exemption from the general prohibition against such activities. In special cases, permission by the government may also be required, which means that the Ministry of the Environment can be considered as a stakeholder too in matters concerning this topic.

The stakeholders also include local residents and operators in areas suffering from extreme occurrences of mosquitoes, as well as conservation organisations such as the Swedish Society for Nature Conservation and WWF.

In December 2013, Mistra EviEM discussed the topic with representatives of the Swedish EPA. Although the topic is fairly well defined, a few clarifications were made:

- Both Swedish and international studies are of interest.
- In the absence of studies of effects on biological diversity, studies of effects on specific non-target organisms are of interest.
- When considering effects on local residents, recreational activities and tourism, the focus should not be on the intervention (use of *Bti*), but rather on exposure (or termination of exposure) to extreme occurrences of mosquitoes.
- Effects on local residents are dominated by psychological factors such as mental well-being and rootedness in place, but long-term and frequent use of mosquito repellents could also cause physiological effects. Declining home prices are a potentially important economic effect.
- When considering livestock and pets, effects of *Bti* use are of primary interest, but effects of the presence of mosquitoes may also be relevant.

Formulation of review questions

Based on the specifications for the report to be produced by the Swedish EPA, a range of conceivable questions for a systematic review have been formulated. When it comes to effects on target organisms, it seems rational to distinguish between the efficacy and the effectiveness of *Bti* as an insecticide and thus consider two different questions:

1a What is the efficacy of *Bti* as an insecticide?

1b How effective is large-scale *Bti* treatment for decreasing the abundance of mosquitoes?

In question 1b, the populations/subjects are areas with extreme mosquito occurrences, the intervention is *Bti* treatment, and the outcome is abundance (or change in abundance) of mosquitoes. The comparator is not specified but could be no intervention or some other intervention.

Questions regarding effects on biodiversity and protected species may also be divided into two separate categories, one concerned with direct effects of *Bti* on non-target organisms, and the other with indirect effects caused by trophic perturbations:

2a What is the direct effect of *Bti* treatment on non-target organisms?

2b What is the effect of *Bti* treatment on biodiversity caused indirectly by changes in the food web?

In question 2b, the populations/subjects and intervention are the same as in question 1b, but the outcome is different and probably needs to be specified (e.g. as species richness, species composition, or mortality or growth of certain non-target species). Additional questions were

3. To what degree does treatment with *Bti* result in resistance among mosquitoes Leder behandling med *Bti* till eutrofiering av vattnet?
4. Does treatment of wetlands with *Bti* result in eutrophication of the water?
5. How persistent is *Bti* in the environment?

Regarding livestock and pets the question may be

6. What is the effect of *Bti* treatment for mosquito control on livestock and pets?

In principle, question 6 is exactly the same as question 2a if livestock and pets are considered as non-target organisms. If we look at the exposure to mosquitoes rather than the intervention itself, the question may be

7. What is the effect of extreme occurrences of mosquitoes (or removing mosquitoes) on livestock and pets?

In question 6 and 7, the population would be livestock and pets within areas associated with extreme occurrences of mosquitoes. In question 7, the intervention can be any activity that changes the mosquito abundance. Questions related to people and society may be formulated as

8. How does the presence of mosquitoes affect human well-being and recreational activities?
9. How does the presence of mosquitoes affect tourism?
10. How does the presence of mosquitoes affect home prices?

The outcomes have to be elaborated further in most of the questions mentioned. In question 9, the outcome could, for example, be the number of visitors or the occupancy rate at hotels and camping grounds, etc.

No specific question has been formulated regarding cumulative effects of repeated treatments during several years. This is because it is assumed that such effects are included among the effects already mentioned, although they may not be possible to detect in short-term studies.

Scientific basis

Searches for related scientific literature have been performed using the Web of Knowledge, Ebscohost, Google and Google Scholar. Different search strings have been used for different questions (see Table 1). Note that the search string "*Bacillus thuringiensis israelensis*" is not appropriate, as it is common to insert various notations for subspecies or serotype before "*israelensis*". The search string "*Bacillus thuringiensis*" generates a large number of hits. However, because there are several insecticides based on Bacillus thuringiensis (Bt) subspecies other than israelensis, many of these hits will be irrelevant. Therefore, the search word "*israelensis*" has been used.

Questions 1 and 2

What is the efficacy of Bti as an insecticide?

How effective is large-scale Bti treatment for decreasing the abundance of mosquitoes?

What is the direct effect of Bti treatment on non-target organisms?

What is the effect of Bti treatment on biodiversity caused indirectly by changes in the food web?

Search string

Search string 1 (Table 1) includes two terms; the first is related to the outcome in question 1 (the target organism), and the other is related to the intervention. The outcome term is rather nonspecific, which in principle makes the search string applicable to several questions. In search string 2, the outcome term is more related to non-target organisms. Search string 1 yielded 1510 hits, 71 of which were reviews.

Review articles

One of the reviews (Boyce et al., 2013) used a systematic approach following the guidelines set forth in the PRISMA statement for systematic reviews and meta-analyses (Liberati et al., 2009). The authors included studies that (i) were conducted in the field where dengue vectors occur naturally, (ii) used *Bti* as the only agent to control dengue vectors, (iii) reported *Bti* formulation and dosing in a clear way, (iv) reported outcomes as indices of immature mosquitoes (i.e. Stegomyia indices, oviposition indices

and/or presence/absence of immature stages of Aedes) and (v) had a minimum follow-up period of 20 days. Fourteen studies (conducted in Southeast Asia, South America and the Caribbean) fulfilled the inclusion criteria. Boyce et al. (2013) concluded that there is evidence for large effects in treated areas; the killing was rapid (<24 h) and residual effects lasted between 2 and 4 weeks. However, results of studies that measured the effectiveness in a community setting were mixed. They did not provide conclusive evidence that *Bti*, when used as a single agent, produced significant reductions of entomological indices. One reason for this could be that the investigators may have failed to identify and treat all potential breeding sites.

Perhaps the most cited paper within this topic is a review by Boisvert and Boisvert (2000) of effects on both target and non-target organisms. The review analysed the results of 75 studies covering approximately 125 families, 300 genera and 400 species. Boisvert and Boisvert (2000) also considered various effect modifiers and pointed out that the dosages required for the control of target organisms, or for effects on non-target organisms, are dependent on the species involved, the type of *Bti* formulations used and/or the environmental conditions of the treated sites. Among non-target species in mosquito habitats, those most frequently reported to be susceptible to *Bti* are chironomids (non-biting midges).

Lacey (2007) reviewed 20 years of mosquito control using *Bti* and concluded that, due to its efficacy and relative specificity, this can be an ideal control agent in integrated programmes, especially where other biological control agents, environmental management, personal protection and judicious use of insecticides are combined.

General reviews within this field, albeit rather brief, have also been authored by Nilsson and Renöfält (2009; in Swedish) and Levanoni and McKie (2010). The latter review provides lists of non-target organisms susceptible to and not susceptible to *Bti*. Because no study in the lists is published after 1999, they seem to be based on the review by Boisvert and Boisvert (2000).

Indirect effects on non-target organisms

With the exception of certain species within the suborder Nematocera, non-target organisms are not susceptible to *Bti* (Lacey, 2007) because they generally do not possess (i) the alkaline gut needed to dissolve the crystals, (ii) the enzymes needed to activate the protoxines, and (iii) the cell membrane receptors needed for binding the toxins. However, there may be indirect effects related to changes in the food web, although rather few studies have investigated them. Changes in insect density and richness have been observed in wetlands in Minnesota, USA (Hershey et al., 1998; Niemi et al., 1999), although no differences between treated and untreated areas were observed in later studies of the same wetlands (Balcer et al., 1999). Effects on insectivorous birds through multitrophic interactions have been reported from the Camargue area in France, where clutch size and fledging success of house martins (Delichon urbicum) were significantly smaller at treated sites relative to control sites (Poulin, 2012; Poulin et al., 2010). Other studies, where black flies were the main target, have failed to indicate any indirect effects on non-target species (Boisvert and Boisvert, 2000; Jackson et al., 2002), but no long-term studies are available.

Question 3

To what degree does treatment with Bti result in resistance among mosquitoes?

In search string 3, a third term has been added in order to specify relevant outcomes. In a recently published review (Ferreira and Neves Lobo Silva-Filha, 2013), it was concluded that there has been no

record of insect resistance to *Bti* in the field, possibly due to the fact that *Bti* crystals contain four different protoxins (Cry4Aa, Cry4Ba, Cry11Aa and Cyt1Aa) that display interactions with multiple midgut receptors.

In the report by Nilsson and Renöfält (2009), the authors mentioned three studies that indicate resistance to *Bti* in the field; one from France (Boyer et al., 2007), one from the USA (Paul et al., 2005), and one from China (Hongyu et al., 2004). (Note that Nilsson and Renöfält (2009) mistakenly write Ayesa et al. instead of Paul et al., and that Hongyu et al. in certain other sources are called Zhang et al.)

Tetreau et al. (2013) question the results of the three studies mentioned above. In the Chinese and American studies, the reference strain was not sampled at about the same time and location as those tested. In these cases, the results could reflect a basal difference of tolerance between the field populations and the reference strain rather than a resistance evolving in the populations treated with *Bti*. Ferreira and Neves Lobo Silva-Filha (2013) compiled resistance ratios measured among populations of different mosquito species from untreated areas, and they concluded that the variation between treated and untreated areas in the Chinese study was similar to the variation in untreated areas. The American study showed higher resistance ratios, but because there are no data from the period before the treatment or from untreated areas in the vicinity, the results are not conclusive.

The French study (Boyer et al., 2007) compared populations sampled in untreated and treated areas. This study did not report the resistance ratio, however, but rather the mortality as a function of time. Tetreau et al. (2013) argued that even in this case, the observed differences can be due to natural tolerance differences between populations rather than to evolved resistance. A more recent study in the same region (Boyer et al., 2012) concluded that resistance ratios were not significantly higher in treated than in untreated areas.

Although the evidence for evolved resistance to *Bti* in the field seems to be weak, it has been shown that resistance to Cry toxins tested separately in bioassays can be significant (Paris et al., 2011; Tetreau et al., 2012b; Tetreau et al., 2013).).

Question 4

Does treatment of wetlands with Bti result in eutrophication of the water?

Search string 4 yielded relatively few studies, and even fewer studies were in some way relevant to the question (most studies focused on the production of *Bti*). No study was found where the authors reported on nutrient mass balances in *Bti*-treated areas. Field experiments by Parkes et al. (2004) showed that removal of black fly larvae by *Bti* resulted in an increased phytoplankton biomass. In contrast, Su and Mulla (1999) found in microcosm studies that *Bti* caused suppressed algal productivity and photosynthesis, which in turn resulted in lower water turbidity and oxygen concentrations in the treatments than in the controls, especially during the hot season. Water in treatments was discernibly clearer than in the controls. An Australian study (Brown et al., 1999) reported that *Bti* did not affect the water quality.

Question 5

How persistent is Bti in the environment?

In this question (search string 5), we are only interested in the intervention (use of *Bti*) and the outcome, which in this case is related to persistence in the environment. Therefore the term related to target organisms has been omitted.

The fate of *Bti* in the environment can be monitored by using three different approaches (Tetreau et al., 2012a). First, its insecticidal activity can be tested by performing bioassays using environmental samples. Second, spores, which usually persist longer than the insecticidal activity, can be detected in field samples by culturing them on petri dishes. Finally, the fate of Cry toxins, which are mainly responsible for the toxicity of *Bti*, has often been studied by using enzyme-linked immunosorbent assays (ELISAs). Tetreau et al. (2012a) argued that only a combination of these three approaches allows the precise monitoring of the persistence of *Bti* in the field. In this way, it may be possible to confirm if recycling of spores is combined with crystal production or not.

The persistence of *Bti* in the field is generally considered to be low (Lacey, 2007). However, some studies have shown that it can persist for a long time and suggest that it can recycle under specific conditions in the environment (Boisvert and Boisvert, 1999; Tilquin et al., 2008; Varjal de Melo-Santos et al., 2009).

Questions 6 and 7

What is the effect of Bti treatment for mosquito control on livestock and pets?

What is the effect of extreme occurrences of mosquitoes (or removing mosquitoes) on livestock and pets?

Considering its mode of action, *Bti* is not expected to be toxic to pets or livestock (or humans). Rather, it has been suggested that *Bti* might be effective as a nematicide and may allow for the development of integrated biological control of zooparasitic nematodes (Sinott et al., 2012; see figure 1).

The mammalian safety of Bacillus thuringiensis-based insecticides was reviewed by Siegel (2001). In summary, numerous laboratory studies have demonstrated that Bt and Bt-based products are noninfectious and are toxic to mammals only at a dose >108 colony forming units (cfu) per mouse (a human equivalent based on the weight of >1011 cfu). Mortality has occurred in some laboratory studies when extremely high doses were administered by invasive routes (intranasal, intracerebral, or intraperitoneal administration). While it has been speculated that immune-compromised individuals are at increased risk for Bt infections (Damgaard et al., 1997; Hernandez et al., 1998), there are no published laboratory data or epidemiology studies that support this (Siegel, 2001).

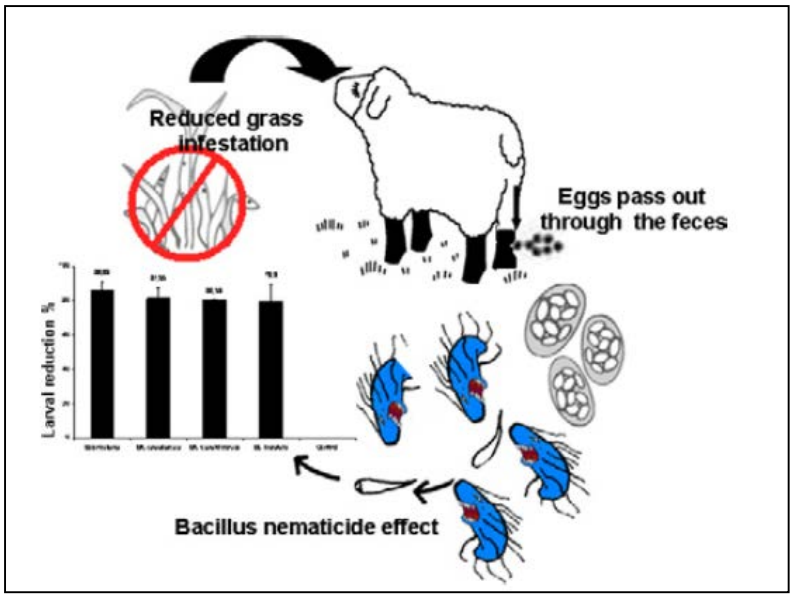

Figure 1. *Bti* used for control of zooparasitic nematodes. From Sinott et al. (2012).

Researchers have expressed concern about the production of Bacillus cereus enterotoxins by Bt isolates. However, despite the existence of enterotoxin genes in Bt, no laboratory safety studies have documented illness in rats and sheep fed Bt products (Siegel, 2001).

In question 7, we are not really concerned with the use of *Bti*, and therefore the intervention term has been substituted by an exposure term related to the target organisms in search string 7. While the populations are the same as in the previous question, an outcome term related to the well-being of the animals has been added.

If the outcome term is omitted, a fairly large number of articles (4792) are found in Web of Knowledge. A significant proportion of these concern various vector-borne diseases usually not occurring in Sweden. In Sweden, the major concern is the nuisance mosquitoes cause the animals. In a statement by veterinarian Mats Scharin (2008-06-03), it is claimed that both sheep and bovine animals get stressed and restless when exposed to extreme occurrences of mosquitoes (see Appendix 2 in the report by Nilsson and Renöfält (2009)). Sheep defend themselves by lying down on the ground and shaking their heads, protecting the less furry body parts. This behaviour may hinder them from eating properly. Bovine animals get attacked by the mosquitoes over large parts of their bodies and they may suffer from loss of blood. They walk around a lot and try to rub off the mosquitoes against trees and bushes and therefore their eating time may be shortened. As a consequence of both blood loss and food shortage, the production of milk of can be reduced.

In Web of Knowledge, only one study investigating milk production in relation to mosquitoes was found. Riha et al. (1979) reported a statistically significant increase in milk production (6.23%) and butterfat percentage (11.8%) by cows treated with a mosquito repellent.

Questions 8, 9 and 10

8. How does the presence of mosquitoes affect human well-being and recreational activities?

The main factors behind human health problems related to non-disease carrying mosquitoes are stress and anxiety caused by the nuisance and limited access to outdoor recreation. Many studies investigate how the occurrence of mosquito-borne diseases affect human health; however, the scientific base is thin when it comes to how the presence of mosquitoes in itself affects human health and well-being. In Johan Hallberg's report Myggen som folkhälsoproblem (Hallberg, 2013), he gave an overview of the available literature.

Based on searches in Ebscohost, Web of Knowledge and Google Scholar, one additional study is worth mentioning: Worobey et al. (2013) examined how the presence of the Asian tiger mosquito affects children's outdoor physical activity. The aim was to investigate whether the presence of mosquitoes can be a barrier to physical activity. The study compared the amount of time that children in areas where mosquito control occurred spent outdoors compared to children in areas where no mosquito control took place. The study concludes that in regions where mosquito control has taken place — and the presence of mosquitoes is substantially lower — children spend on average 129 minutes more outdoors per day. The number of children in the study was relatively low; at the first measurement occasion, there were 4 children in the area where mosquito control took place and 8 children in an area where no mosquito control took place, and 18 and 8 respectively on the second occasion.

Another study has obtained similar results for adults. Shepard et al. (2012) studied some 300 households and found that the differences between actual and potential hours of yard and porch activities were 1.88 hours/week (only abstract available). Although there is limited scientific evidence on the effect of mosquitoes on human well-being, there are countless reports from individuals of the discomfort that mosquitoes create. This fact is supported by several studies that have shown that the willingness to pay exceeds the cost of mosquito control (Hirsch and Becker, 2009; Ofiara and Allison, 1986). Also, Dickinson and Paskewitz (2012) reported that among homeowners in Madison, Wisconsin, USA, demands for mosquito control were more related to the "nuisance factor" than the "disease factor".

9. How does the presence of mosquitoes affect tourism?

Few studies examine how the presence of mosquitoes affects tourism. Soutukorva et al. (2013) gave a comprehensive account of what little evidence there is on the subject, and also of their own findings. What can be noted is that more research is needed in this area, especially of how tourism is affected by the presence of mosquitoes locally.

10. How does the presence of mosquitoes affect home prices?

Prior to the revision of property taxation for 2014, an evaluation of the situation surrounding Dalälven River was carried out by the Swedish Tax Authority. Due to the low number of sales in the area, an appreciator with good local knowledge was asked to assess the property values in the area. This evaluation was followed by a significant reduction in the property taxation. The benchmark for a normal single-family home in the area was reduced from 120,000 SEK to 80,000 SEK. Although the presence of mosquitoes was not singled out in the evaluation as a factor behind the lowering of property values, this was actually the main reason for the reduction according to the Swedish Tax Authority (Per Husberg, Swedish Tax Autority, personal communication, January 28, 2014).

Table 1. Search strings used in Web of Knowledge (field=topic) and Ebscohost. Number of hits in brackets indicates number of articles published 2010-2014.

Question	Seach string	Hits
1	Web of knowledge: (mosquito* OR Anopheles OR Aedes OR Culex OR Culiseta OR Limatus OR Uranotaenia OR Psorophora OR Mansonia OR Armigeres OR Trichoprospon OR Coquillettidia OR Tripteroides OR larv*) and (*bti* OR israelensis OR vectobac)	1510 (282)
2	Web of knowledge: (Non*target OR indirect OR vertebrate OR invertebrate OR bird OR fish OR diptera OR amphibian$ OR crustacean$ OR *worm$ OR nematoda* OR mollus* OR insect$) and (*bti* OR israelensis OR vectobac)	1079 (235)
3	Web of knowledge: **1** and (resistan* OR "positive selection")	335 (87)
4	Web of knowledge: **1** and (eutroph* OR nutrient* OR "water quality")	28 (5)
5	Web of knowledge: (*bti* OR israelensis OR vectobac) and (persisten* OR "residual effect" OR fate)	138 (37)
6	Web of knowledge: (*bti* OR israelensis OR vectobac) and (pet OR dog OR cat OR livestock OR cattle OR bovine OR cow OR sheep OR goat OR horse OR pig OR swine)	81 (17)
7	Web of knowledge: (mosquito* OR Anopheles OR Aedes OR Culex OR Culiseta OR Limatus OR Uranotaenia OR Psorophora OR Mansonia OR Armigeres OR Trichoprospon OR Coquillettidia OR Tripteroides) and (pet OR dog OR cat OR livestock OR cattle OR bovine OR cow OR sheep OR goat OR horse OR pig OR swine) and (stress* OR annoy* OR nuisance)	78 (26)
8	Ebscohost: (mosquito* and public health)	5555
8	Ebscohost: (mosquito* and outdoor activit*)	144
8	Web of knowledge: ((mosquito* OR Anopheles OR Aedes OR Culex OR Culiseta OR Limatus OR Uranotaenia OR Psorophora OR Mansonia OR Armigeres OR Trichoprospon OR Coquillettidia OR Tripteroides) and outdoor activit*)	62
8	Web of knowledge: ((mosquito* OR Anopheles OR Aedes OR Culex OR Culiseta OR Limatus OR Uranotaenia OR Psorophora OR Mansonia OR Armigeres OR Trichoprospon OR Coquillettidia OR Tripteroides) and public health)	1321
9	Ebscohost: (mosquito* and tourism)	76
9	Web of knowledge: ((mosquito* OR Anopheles OR Aedes OR Culex OR Culiseta OR Limatus OR Uranotaenia OR Psorophora OR Mansonia OR Armigeres OR Trichoprospon OR Coquillettidia OR Tripteroides) and tourism)	29

Swedish reports (grey literature)

This section provides an overview of recent Swedish grey literature.

Nilsson and Renöfält (2009) reviewed the results of the evaluation programme for the Swedish mosquito control activities along the lower reaches of Dalälven River. In addition to a comprehensive list of reports and scientific articles emanating from that programme, the report provides a general review on *Bti* and its effects on target and non-target organisms.

In 2010, the Swedish EPA was commissioned by the Swedish government to report on how they worked to handle identified problems with mosquitoes in the lower Dalälven River region. The report (Naturvårdsverket, 2010) provides a comprehensive background on the problems and situation in the region.

A research group at the Swedish University of Agricultural Sciences (SLU) was commissioned by the Swedish EPA to develop alternative approaches for monitoring the effects of the mosquito control agent *Bti* on ecosystems of the Dalälven River catchment. In their report (McKie and Goedkoop, 2010), the authors provide a general introduction to *Bti*, non-target organisms and biomonitoring. Appendix I (Levanoni and McKie, 2010) is a literature review, and appendix II is a questionnaire regarding use of *Bti* that has been filled out by mosquito control agencies in Canada (City of Winnipeg) and in France (Le Tour du Valat).

The authorization of VectoBac G by the Swedish Chemicals Agency is based on a Product Assessment Report (KEMI, 2010a,b) in which both Swedish and international studies are reviewed.

In 2010, Gävleborg the County Administrative Board reported to the Swedish government on a regional strategic plan on how to cope with mosquitoes, and in 2013, they reported on efforts that had been made according to the plan. The final report (Lundqvist et al., 2013) was accompanied by 21 appendices, some of which are research reports that are relevant to some of the questions discussed in this pilot study:

- Appendix 7 (Gerhard et al., 2013) reported briefly on the use of *Bti* in a few countries in Europe and North America.
- Appendix 8 (Soutukorva et al., 2013) reported on macroeconomic aspects, e.g. willingness-to-pay studies, and provided information relevant to question 9.
- Appendix 10 (Hallberg, 2013) elaborated on public health issues relevant to question 8.
- Appendix 19 (Ojala and Lidskog, 2013) is a survey of the views concerning mosquitoes of people living at Lake Björken (relevant to question 8).

In a Master's thesis from the Department of Psychology at Uppsala University, the graduate student investigated the perception of the home environment and mental health of people exposed to dense mosquito populations (Nordström, 2010). The thesis is relevant to question 8.

Fulfilment of selection criteria

As a summary of the preceding sections, this section presents tables showing the extent to which the suggested primary review questions fulfil the criteria for selection of systematic review topics that have been approved by the EviEM Executive Committee. Table 1 shows the mandatory criteria for all questions, and table 2 shows the optional criteria for questions where all the mandatory criteria are fulfilled.

Table 1. Fulfilment of mandatory criteria for questions 1-10.

Mandatory criterion	**1a**	**1b**	**2a**	**2b**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Deals with conditions in the natural environment	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Relevant to the situation in Sweden	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Well-defined, conceptually clear and reasonably limited in scope	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Deals with problem descriptions or countermeasures whose scientific support is insufficient, disputed or incompletely known	N	Y	N	Y	N	Y	Y	N	Y	Y	Y	Y
Covered in the scientific literature (or by other investigations) to such an extent that a systematic assessment can be implemented	Y	Y	Y	N	Y	N	Y	N	N	N	N	N

Table 1. Fulfilment of optional criteria for questions 1b and 5.

Optional criterion	Y/N	Comments
Controversial and/or the subject of great public attention	Y	Very important to people living in areas with dense mosquito populations
Seen as environmental policy issues of high concern	Y	The Swedish EPA has been commissioned to report to the government.
Deals with new forms of environmental pressures, changes or actions	Y	A *Bti*-based insecticide has been used along the lower Dalälven River since 2002.
Deals with environmental disturbances or mitigation efforts that affect great natural values and/or extensive parts of the country	Y	*Bti* treatments are carried out close to and within protected areas
Deals with measures that are particularly costly or resource-demanding in some other respect	?	
Deals with actions that in some respects are beneficial to the environment but may be unfavourable in other respects	N	
Deals with environmental problems currently treated by a variety of alternative measures	Y	Other suggested measures to control mosquitoes near Dalälven River include, for example, changed regulation of the river and grazing by livestock to keep the landscape open.

Conclusion

Bti-based insecticides have been used in several countries on all continents since the early 1980s. There are numerous studies on several aspects of *Bti*, and quite a few review articles have been published too. Although the knowledge base is quite large within this topic, there are some knowledge gaps. Below is a summary of what is (1) thoroughly investigated in many studies and generally accepted, (2) investigated in just a few studies and where more research is needed, and (3) fairly extensively investigated with mixed results.

Thoroughly investigated questions with generally accepted answers

It is well established that the efficacy of *Bti*-based products for controlling mosquito larvae is high. This means that *Bti* has been shown to be effective in killing mosquito larvae in laboratory experiments or within well-delineated breeding sites in the field.

It is also fairly well established that the selectivity of *Bti* is high. The selectivity can be explained in terms of the mode of action of *Bti*, which is complex but relatively well understood. The largest group of non-target organisms that have been shown to be susceptible to direct effects of *Bti* consists of various chironomid species. Black flies (Simuliidae) are also very susceptible to *Bti*, but these are often considered target organisms.

Despite several investigations, only a few studies have indicated any resistance to *Bti* in the field, and the results of those studies have been questioned on methodological grounds. In laboratory experiments, only a slight resistance to *Bti* has been achieved, whereas a stronger resistance to individual *Bti* protoxins has evolved.

It seems to be well established that *Bti* is non-infectious and that it is toxic to mammals (including humans) only at very high doses. Despite the existence of enterotoxin genes in Bt isolates, no laboratory safety studies have documented illness in rats and sheep fed Bt products.

Thus, questions with generally accepted answers may include:

What is the efficacy of Bti as an insecticide?

What is the direct effect of Bti treatment on non-target organisms?

To what degree does treatment with Bti result in resistance among mosquitoes?

What is the effect of Bti treatment for mosquito control on livestock and pets?

Rarely studied questions with poorly known answers

Reduction or removal of one species in a system may induce negative or positive effects on other species, which in turn can influence, for example, species richness or species dominance in the system. However, studies investigating such effects where *Bti* has been used (or indirect effects on non-target organisms resulting from changes in the food web) are quite rare. This is especially true for long-term studies.

Few studies have touched upon the relationships between *Bti* treatment and water quality. In Web of Knowledge, no study could be found where the nutrient budget was investigated in *Bti*-treated areas.

Also, relatively little research has been conducted on the effects of *Bti* on human well-being and recreational activities, tourism and economy. A few Swedish reports (Hallberg, 2013; Nordström, 2010; Ojala and Lidskog, 2013; Soutukorva et al., 2013) contribute significantly to the state of knowledge in the field. Furthermore, some studies have shown that both children and adults spend less time outdoors in mosquito-rich areas, and that the willingness to pay for mosquito control is relatively high, as mentioned above.

Few studies have investigated stress-related effects of exposure to mosquitoes on livestock and pets. One Czech study reported a statistically significant increase in milk production and butterfat percentage among cows treated with a mosquito repellent.

Thus, questions where more research is needed include:

What is the effect of Bti treatment on biodiversity caused indirectly by changes in the food web?

Does treatment of wetlands with Bti result in eutrophication of the water?

What is the effect of extreme occurrences of mosquitoes (or removing mosquitoes) on livestock and pets?

How does the presence of mosquitoes affect human well-being and recreational activities?

How does the presence of mosquitoes affect tourism?

How does the presence of mosquitoes affect home prices?

Fairly extensively investigated with mixed results

Although the efficacy of *Bti* for controlling mosquito larvae has proved to be high, the effectiveness of *Bti* as a single agent for controlling mosquitoes on a larger scale (community or landscape level) has been questioned. Boyce et al. (2013) found no conclusive evidence for the latter when conducting a systematic review focusing on dengue vectors. However, given the worldwide use of *Bti* and the number of studies on *Bti* effectiveness, it should be possible to conduct a systematic review similar to that of Boyce et al. (2013), but with a focus more specific to Swedish conditions.

The residual insecticidal effect of *Bti* is generally considered to be fairly short (weeks to months). However, several studies have shown that *Bti* spores can persist much longer, and some studies have shown that recycling of the spores can occur (Tetreau et al., 2012a). The amount of studies specifically addressing this question is not huge, but it may be sufficient for a systematic review.

Thus, the following questions may be viable for a systematic review:

What is the effectiveness of large-scale Bti treatment for decreasing the abundance of mosquitoes?

How persistent is Bti in the environment?

References

Balcer, M.D., Schmude, K.L., Snitgen, J., 1999**. Long-term Effects of the Mosquito Control Agents *Bti* (Bacillus Thuringiensis Israelensis) and Methoprene on Non-target Macroinvertebrates in Wetlands in Wright County**, Minnesota (1997-1998). LSRI, Wisconsin.

Boisvert, M., 2007**. Utilization of Bacillus thuringiensis var. israelensis (*Bti*)-Based Formulations for the Biological Control of Mosquitoes in Canada**, in: Côté, J.C., Otvos, I.S., Schwartz, J.L., Vincent, C. (Eds.), 6th Pacific Rim Conference on the Biotechnology of Bacillus thuringiensis and its Environmental Impact, Victoria BC, Canada, Oct 30 - Nov 3, 2005, pp. 87-93.

Boisvert, M., Boisvert, J., 1999. **Persistence of toxic activity and recycling of Bacillus thuringiensis var. israelensis in cold water: Field experiments using diffusion chambers in a pond**. Biocontrol Science and Technology 9, 507-522.

Boisvert, M., Boisvert, J., 2000. **Effects of Bacillus thuringiensis var. israelensis on target and nontarget organisms: a review of laboratory and field experiments**. Biocontrol Science and Technology 10, 517-561.

Boyce, R., Lenhart, A., Kroeger, A., Velayudhan, R., Roberts, B., Horstick, O., 2013. **Bacillus thuringiensis israelensis (*Bti*) for the control of dengue vectors: systematic literature review**. Tropical Medicine & International Health 18, 564-577.

Boyer, S., Paris, M., Jego, S., Lemperiere, G., Ravanel, P., 2012. **Influence of insecticide Bacillus thuringiensis subsp israelensis treatments on resistance and enzyme activities in Aedes rusticus larvae (Diptera: Culicidae)**. Biological Control 62, 75-81.

Boyer, S., Tilquin, M., Ravanel, P., 2007. **Differential sensitivity to Bacillus thuringiensis var. Israelensis and temephos in field mosquito populations of Ochlerotatus cataphylla (Diptera : Culicidae): Toward resistance?** Environmental Toxicology and Chemistry 26, 157-162.

Brown, M.D., Thomas, D., Mason, P., Greenwood, J.G., Kay, B.H., 1999. **Laboratory and field evaluation of the efficacy of four insecticides for Aedes vigilax (Diptera : Culicidae) and toxicity to the nontarget shrimp Leander tenuicornis (Decapoda : Palaemonidae)**. Journal of Economic Entomology 92, 1045-1051.

Damgaard, P.H., Granum, P.E., Bresciani, J., Torregrossa, M.V., Eilenberg, J., Valentino, L., 1997. **Characterization of Bacillus thuringiensis isolated from infections in burn wounds**. Fems Immunology and Medical Microbiology 18, 47-53.

Dickinson, K., Paskewitz, S., 2012. **Willingness to Pay for Mosquito Control: How Important Is West Nile Virus Risk Compared to the Nuisance of Mosquitoes?** Vector-Borne and Zoonotic Diseases 12, 886-892.

Ferreira, L.M., Neves Lobo Silva-Filha, M.H., 2013. **Bacterial larvicides for vector control: mode of action of toxins and implications for resistance**. Biocontrol Science and Technology 23, 1137-1168.

Gerhard, K., Iwarsson, M., Tunón, H., 2013. **Sammanställning och bedömning av myggbegränsningsmetoder i Sverige och andra länder (in Swedish)**. Länsstyrelsen i Gävleborg, Rapport 2013:12, Gävle, Sweden.

Goldberg, L.J., Margalit, J., 1977. **Bacterial spore demonstrating rapid larvicidal activity against anopheles-sergentii, uranotaenia-unguiculata, culex-univitattus, aedes-aegypti and culex-pipiens**. Mosquito News 37, 355-361.

Gonsalves, L., Law, B., Webb, C., Monamy, V., 2013. **Foraging Ranges of Insectivorous Bats Shift Relative to Changes in Mosquito Abundance**. Plos One 8.

Hallberg, J., 2013. **Myggen som folkhälsoproblem (in Swedish)**. Länstyrelsen i Gävleborg, Rapport 2013:23, Gävle, Sweden.

Hernandez, E., Ramisse, F., Ducoureau, J.P., Cruel, T., Cavallo, J.D., 1998. **Bacillus thuringiensis subsp. konkukian (serotype H34) superinfection: Case report and experimental evidence of pathogenicity in immunosuppressed mice**. Journal of Clinical Microbiology 36, 2138-2139.

Hershey, A.E., Lima, A.R., Niemi, G.J., Regal, R.R., 1998. **Effects of Bacillus thuringiensis israelensis (*Bti*) and methoprene on nontarget macroinvertebrates in Minnesota wetlands**. Ecological Applications 8, 41-60.

Hirsch, H., Becker, N., 2009. **Cost-benefit analysis of mosquito control operations based on microbial control agents in the upper Rhine valley (Germany)**. European Mosquito Bulletin 27, 47-55.

Hongyu, Z.Y., Yang, C.J., Huang, J.Y., Lu, L., 2004. **Susceptibility of field populations of Anopheles sinensis (Diptera : Culicidae) to Bacillus thuringiensis subsp israelensis**. Biocontrol Science and Technology 14, 321-325.

Jackson, J.K., Horwitz, R.J., Sweeney, B.W., 2002. **Effects of Bacillus thuringiensis israelensis on black flies and nontarget macroinvertebrates and fish in a large river**. Transactions of the American Fisheries Society 131, 910-930.

Karlsson, M., Terstad, J., 2012. **Naturskyddsföreningens svar på remiss rörande ansökan om spridning av bekämpningsmedel Vectobac G i Deje, Forshaga kommun respektive Nedre Dalälvsområdet (in Swedish)**, Swedish Society for Nature Conservation.

KEMI, 2010a. **VectoBac G. Product Assessment Report – Change of condition (F-3422-221-09)**, Stockholm, Sweden.

KEMI, 2010b. **VectoBac G. Product Assessment Report (F-3422-221-09)**, Stockholm, Sweden.

Lacey, L.A., 2007. **Bacillus thuringiensis serovariety israelensis and Bacillus sphaericus for mosquito control**. Journal of the American Mosquito Control Association 23, 133-163.

Levanoni, O., McKie, B., 2010. **Assessing the effects of the mosquito control agent *Bti* (Bacillus thuringiensis serovar israelensis): a literature review of Swedish and international studies**, in: McKie, B., Goedkoop, W. (Eds.), Final Report, Development of alternative

approaches for monitoring the effects of the mosquito control agent *Bti* on ecosystems of the Dalälven catchment. Swedish University of Agricultural Sciences, Uppsala, Sweden.

Liberati, A., Altman, D.G., Tetzlaff, J., Mulrow, C., Gotzsche, P.C., Ioannidis, J.P.A., Clarke, M., Devereaux, P.J., Kleijnen, J., Moher, D., 2009. **The PRISMA Statement for Reporting Systematic Reviews and Meta-Analyses of Studies That Evaluate Health Care Interventions: Explanation and Elaboration**. Plos Medicine 6.

Lundqvist, A.-C., Widemo, M., Lindquist, I., 2013. **Förslag till hur myggproblemet vid Nedre Dalälven kan hanteras på lång sikt (in Swedish)**. Länsstyrelsen Gävleborg, Rapport 500-8033-13, Gävle, Sweden.

McKie, B., Goedkoop, W., 2010. **Development of alternative approaches for monitoring the effects of the mosquito control agent *Bti* on ecosystems of the Dalälven catchment**. Department of Aquatic Sciences & Assessment, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Naturvårdsverket, 2010. **Myggproblemen i Nedre Dalälvsområdet 2010 -Så arbetar Naturvårdsverket med frågan (in Swedish)**. Naturvårdsverket Rapport NV-03659-10, Stockholm, Sweden.

Niemi, G.J., Hershey, A.E., Shannon, L., Hanowski, J.M., Lima, A., Axler, R.P., Regal, R.R., 1999. **Ecological effects of mosquito control on zooplankton, insects, and birds**. Environmental Toxicology and Chemistry 18, 549-559.

Nilsson, C., Renöfält, B., 2009. **Mygg och *Bti* i nedre Dalälven, Utvärdering av ett vetenskapligt uppföljningsprogram (in Swedish with English summary)**. Naturvårdsverket, Rapport 6305, Stockolm, Sweden.

Nordström, A., 2010. **Upplevelser av boendemiljö och psykisk hälsa hos boende i områden med förekomst av översvämningsmygg (in Swedish)**, Department of Psychology. Upsala University, Uppsala Sweden.

Ofiara, D.D., Allison, J.R., 1986. **A comparison of alternative mosquito abatement methods using benefit-cost-analysis**. Journal of the American Mosquito Control Association 2, 522-528.

Ojala, M., Lidskog, R., 2013. **Mygg och människor vid sjön Björken: Upplevelser av myggsituationen och attityder till bekämpningsåtgärder (in Swedish)**. Länsstyrelsen i Gävleborg, Rapport 2013:19, Gävle, Sweden.

Paris, M., Tetreau, G., Laurent, F., Lelu, M., Despres, L., David, J.-P., 2011. **Persistence of Bacillus thuringiensis israelensis (*Bti*) in the environment induces resistance to multiple *Bti* toxins in mosquitoes**. Pest Management Science 67, 122-128.

Parkes, A.H., Kalff, J., Boisvert, J., Cabana, G., 2004. **Feeding by black fly (Diptera : Simuliidae) larvae causes downstream losses in phytoplankton, but not bacteria**. Journal of the North American Benthological Society 23, 780-792.

Paul, A., Harrington, L.C., Zhang, L., Scott, J.G., 2005. **Insecticide resistance in Culex pipiens from New York**. Journal of the American Mosquito Control Association 21, 305-309.

Poulin, B., 2012. **Indirect effects of bioinsecticides on the nontarget fauna: The Camargue experiment calls for future research**. Acta Oecologica-International Journal of Ecology 44, 28-32.

Poulin, B., Lefebvre, G., Paz, L., 2010. **Red flag for green spray: adverse trophic effects of *Bti* on breeding birds**. Journal of Applied Ecology 47, 884-889.

Ramírez-Lepe, M., Ramírez-Suero, M., 2012. **Biological Control of Mosquito Larvae by Bacillus thuringiensis subsp. israelensis**, in: Perveen, F. (Ed.), Insecticides - Pest Engineering. InTech, Rijeka, Croatia, pp. 239-264.

Riha, J., Minar, J., Lamatova, Z., Matouskova, O., 1979. **Economic importance of the prevention of losses caused by the blood-sucking diptera in grazing cattle**. Veterinarni Medicina 24, 275-283.

Shepard, D., Halasa, Y., Wittenberg, E., Fonseca, D., Farajollah, A., Healy, S., Gaugler, R., Bartlett-Healy, K., Strickman, D., Clark, G., 2012. **Cost-benefit analysis of an area-wide pest management program to control Asian tiger mosquito in New Jersey**. USDA, http://www.ars.usda.gov/research/publications/Publications.htm?seq_no_115=288175.

Siegel, J.P., 2001. **The mammalian safety of Bacillus thuringiensis-based insecticides**. Journal of Invertebrate Pathology 77, 13-21.

Sinott, M.C., Cunha Filho, N.A., Castro, L.L.D., Lorenzon, L.B., Pinto, N.B., Capella, G.A., Leite, F.P.L., 2012. **Bacillus spp. toxicity against Haemonchus contortus larvae in sheep fecal cultures**. Experimental Parasitology 132, 103-108.

Soutukorva, Å., Johansson, K., Hasselström, L., Cole, S., Remvig, H., Kriström, B., 2013. **Samhällsekonomisk analys av myggproblemets kostnader (in Swedish)**. Länsstyrelsen i Gävleborg, Rapport 2013:16, Gävle, Sweden.

Su, T.Y., Mulla, M.S., 1999. **Microbial agents Bacillus thuringiensis ssp israelensis and Bacillus sphaericus suppress eutrophication, enhance water quality, and control mosquitoes in microcosms**. Environmental Entomology 28, 761-767.

Tetreau, G., Alessi, M., Veyrenc, S., Perigon, S., David, J.-P., Reynaud, S., Despres, L., 2012a. **Fate of Bacillus thuringiensis subsp israelensis in the Field: Evidence for Spore Recycling and Differential Persistence of Toxins in Leaf Litter**. Applied and Environmental Microbiology 78, 8362-8367.

Tetreau, G., Bayyareddy, K., Jones, C.M., Stalinski, R., Riaz, M.A., Paris, M., David, J.-P., Adang, M.J., Despres, L., 2012b. **Larval midgut modifications associated with *Bti* resistance in the yellow fever mosquito using proteomic and transcriptomic approaches**. Bmc Genomics 13.

Tetreau, G., Stalinski, R., David, J.-P., Despres, L., 2013. **Monitoring resistance to Bacillus thuringiensis subsp israelensis in the field by performing bioassays with each Cry toxin separately**. Memorias Do Instituto Oswaldo Cruz 108, 894-900.

Tilquin, M., Paris, M., Reynaud, S., Despres, L., Ravanel, P., Geremia, R.A., Gury, J., 2008. **Long Lasting Persistence of Bacillus thuringiensis Subsp israelensis (*Bti*) in Mosquito Natural Habitats**. Plos One 3.

Varjal de Melo-Santos, M.A., de Araujo, A.P., Maranhao Rios, E.M., Regis, L., 2009. **Long lasting persistence of Bacillus thuringiensis serovar. israelensis larvicidal activity in Aedes aegypti (Diptera: Culicidae) breeding places is associated to bacteria recycling**. Biological Control 49, 186-191.

Worobey, J., Fonseca, D.M., Espinosa, C., Healy, S., Gaugler, R., 2013. **Child outdoor physical activity is reduced by prevalence of the asian tiger mosquito, aedes albopictus**. Journal of the American Mosquito Control Association 29, 78-80.

Mistra EviEM Pilot Studies

PS1 Pilot Study: **How are fluxes of greenhouse gases between boreal forest ecosystems and the atmosphere affected by uneven-aged forestry?** (October 2012)

PS2 Pilot Study: **What is the effect of pharmaceutical residues in water on aquatic biota?** (February 2013)

PS3 Pilot Study: **Is thiamine deficiency a significant cause of declining bird populations in the Baltic Sea area?** (November 2013)

Mistra EviEM conducts systematic reviews of environmental issues identified as important by public agencies and other stakeholders. These provide an overall assessment of the state of scientific knowledge and help to improve the basis for environmental decision-making in Sweden.

All systematic reviews are preceded by a pilot study. If such a study indicates that the topic is not suitable for systematic review, the process ends with the pilot study being published in this series instead.

EviEM
Mistra Council for Evidence-based Environmental Management

Address:
Royal Swedish Academy of Sciences
Box 50005, SE-104 05 Stockholm, Sweden

Visit/Deliveries:
Lilla Frescativägen 4A, SE-114 18 Stockholm, Sweden

Phone: 08-673 95 00 | Email: info@eviem.se | **www.eviem.se**