# A Review and Update of the Report "Environmental and health impacts of *Bacillus thuringiensis israelensis*" 1998 by Travis R. Glare and Maureen O'Callaghan

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**Report for**
**New Zealand Ministry of Health**
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## Contents

## Abbreviations:

Bt - *Bacillus thuringiensis*; Bts - *Bacillus thuringiensis* strains (plural); Bti - *Bacillus thuringiensis israelensis*; Btk - *Bacillus thuringiensis kurstaki*; Bs - *Bacillus sphaericus*; methoprene – S-methoprene

## 1. Summary

Glare and O'Callaghan (1998) developed a report for the Ministry of Health on the environmental and health impacts associated with the use of *Bacillus thuringiensis israelensis* (Bti) for control and eradication of mosquitoes in New Zealand. This report along with a companion report on S-methoprene (Glare and O'Callaghan 1999), was developed in response to recent introductions of non-indigenous mosquito species that can serve as vectors for disease in humans. These pesticides were not registered for use as mosquito control agents in New Zealand and were thought at the time to be the best choices for control and/or eradication programmes in the country. Both assessments were extremely well-done and covered the known literature of the time. The general conclusions of the report by Glare and O'Callaghan (1998) were that Bti has been developed into many products for the control of dipteran pests, including mosquitoes (Culicidae), blackflies (Simuliidae) and midges (Chironomidae). These products have been used extensively in control programmes for mosquitoes and biting flies in various parts of the world. Bt strains occur naturally in New Zealand. The Bti strains used in pest control have a proven history of environmental safety. Bti is highly selective and thus has very little potential to cause damage to populations of nontarget organisms. Bti exhibits short environmental persistence. There is the possibility that the genes encoded for some of the toxic proteins of Bt residing on extrachromosomal DNA (plasmids) may be exchanged between strains and species by conjugation and/or transformation. However, no example of an unexpected pathogenic organism being developed in the field has been demonstrated. Some insects, especially Lepidoptera, have developed resistance to Bt strains. However, no examples of resistance to Bti in field populations of mosquitoes have been documented. Even though over 40 tons of Bti have been applied in west Africa alone, no indications of human health or nontarget effects have been reported. Bti appears to pose significantly less of a risk than other pesticides used for mosquito control and eradication programmes. Much of the new literature on nontarget effects still indicates that Bti is one of the least environmentally damaging pesticides used for mosquito control. Several new reports have shown that large declines in insect biomass can occur after long-term use of Bti in freshwater wetlands. However, no evidence for permanent damage to ecosystem function has been found. Organisms that utilised insects for food, adapted to the declines and either switched to other food sources or travelled (birds) outside of the treated zones to acquire insects. It is my opinion that the conclusions reached by Glare and O'Callaghan in 1998 are still valid today and I would recommend that Bti be used for control and eradication of introduced mosquito species in New Zealand, particularly if it is used on a rotation basis with methoprene. The justification for this recommendation is that the alternative control agents, other than Bs, are organophorous insecticides like temephos that are very broad-spectrum neurotoxins that pose a much more serious risk to the environment, human and animal health than Bti. New pesticides, such as spinosad (a natural product) and fipronil (a synthetic neurotoxin) have shown promise as controls for mosquitoes, but the potential impacts of these products on human health and nontarget organisms is not presently known because they haven't been used in wide-scale control/eradication programmes to date.

## 2. Introduction

It is my intention to update the literature on Bti in this report. This report should be read in conjunction with the original Glare and O'Callaghan report which is available at www.moh.govt.nz. For ease of comparison to the original document (Glare and O'Callaghan 1998), each section will be numbered exactly as the original document. However, new additional literature was not necessarily found for each of the sections of the original report and therefore some sections will only contain a statement that new literature has not been published since the original report. Three new sections have been added to this document that did not appear in the original report. The first is section 8.4 and deals with effects of Bti on birds. The second is section 8.5 and deals with Bti effects on algae. The third new section is unnumbered and is located just before the Discussion and Conclusions section. This section is entitled "Use of Bti in Control and Eradication Programmes".

A search of the Biological Abstracts database and the Agricola database with the key word *Bacillus thuringiensis israelensis* from 1997 to the present produced 81 new references since the 1998 report by Glare and O'Callaghan. Of these 81 documents, 79 were used in this report while two of the articles were not pertinent to the subject of Bti use for mosquito control. Seven additional papers related to this subject were also used in this report.

### *2.1 Background*

The establishment of non-indigenous mosquitoes in New Zealand poses a threat to human health and the environment (Frampton 2004). The discovery of the southern saltmarsh mosquito, *Ochlerotatus camptorhynchus* in 1998 near Napier in the North Island and subsequent detections in other areas of the North Island and in the Wairau estuarine area in the northern part of the South Island has led to an eradication campaign for this species (Frampton 2004). Based on environmental and health impact assessments of S-methoprene and *Bacillus thuringiensis israelensis* (Bti) by Glare and O'Callaghan (1998, 1999), methoprene and to a more limited extent, Bti, have been used as the control agents in the eradication campaign.

### *2.2 Discovery of Bacillus thuringiensis israelensis*

No new information on the discovery of Bti has been published since the original report by Glare and O'Callaghan (1998).

## 3. Characterisation of *Bacillus thuringiensis israelensis*

**The classification of Bti was stated by Glare and O'Callaghan (1998) to be difficult because of the close genetic relationship between Bt and *B. cereus*, *B. anthracis* and *B. mycoides*. This is still the case today.**

### *3.1 Bacillus thuringiensis taxonomy*

The taxonomic description of Bt by Glare and O'Callaghan (1998) is still valid today. As stated in the original document by Glare and O'Callaghan (1998), Bti is a gram positive, rod-shaped, spore forming bacterium which often has insecticidal properties. *B. thuringiensis* belongs to the *Bacillus cereus* complex which also includes *B. cereus*, *B. anthracis* and *B. mycoides*.

### *3.2 Characterisation*

The characterisation of Bt described by Glare and O'Callaghan (1998) has not changed since the original report.

### *3.3. Mode of action*

Our understanding of the mode of action of Bti has not changed since the report by Glare and O'Callaghan (1998). In general Bt contains spores and parasporal bodies that encase toxic proteins. Once ingested by organisms with an alkaline gut pH, the crystals dissolve and destroy the midgut epithelium resulting in death. However, some new information (see below) regarding the mode of action of Bti on other organisms has been published.

Adult, female *Stegomyia aegypti* (formerly *Aedes aegypti*), some of which had been exposed to sublethal concentrations of Bti as larvae, were fed on chickens with gametocytaemia of the malaria parasite, *Plasmodium gallinaceum* (Kala and Gunasekaran 1999). Parasite development was monitored through dissection of the mosquitoes immediately after they were fed and on days 1, 7 and 9, to check for the presence of gametocytes, ookinetes, oocysts and sporozoites, respectively. There were no significant differences between Bti–exposed and unexposed mosquitoes for the presence of gametocytes and ookinetes and the density of these parasite stages. This indicated that ingestion of gametocytes and development of ookinetes did not appear to be affected by Bti exposure. However, Bti-treated mosquitoes were significantly less likely to carry oocysts than mosquitoes not exposed to Bti. Bti-exposed mosquitoes that were oocyst-positive carried significantly fewer oocysts than the unexposed insects, indicating that Bti induced parasite loss during development of oocysts. This in turn resulted in fewer mosquitoes with sporozoites in the Bti-exposed group showing that mosquitoes surviving Bti treatment did not fully support development of the parasite responsible for malaria.

*Culex quinquefasciatus* larvae were exposed to Bs and Bti to study ultrastructural changes in the midgut epithelial cells (Poopathi et al. 1999a). Bs disturbed the structural integrity of midgut epithelial cells. Gross ultrastructural changes in the microvilli, formation of numerous vacuoles in the cytoplasm, appearance of cytoplasmic filaments, mitochondrial swelling, discernible cisterna and progressive number of lysosomes by a rapid multiplication process were also observed. Bti exposure resulted in microvilli appearing to bubble followed by a total collapse in cytoplasm.

Cavados et al. (2004) investigated the mode of action of Bti endotoxins on the larvae of the black fly, *Simulium pertinax*, a pest species common in Brazil. Light and electron microscope observations revealed that damage of the larvae midgut epithelium occurred after exposure. In particular, midgut columnar cell vacuolization occurred, microvilli were damaged and epithelium cell contents passed into the midgut lumen resulting in cell death.

## 4. Natural occurrence and use of *Bt* in New Zealand

**Bt occurs naturally in many parts of the world including New Zealand.**

### *4.1 Presence of Bt in New Zealand*

The section on the presence of Bt in New Zealand is still valid.

### *4.2 Occurrence and role in the environment*

The section on occurrence and role in the environment by Glare and O'Callaghan (1998) is still valid. One new study was found dealing with this subject (see below).

Surveys were conducted to determine whether Bti present in natural and artificial containers in Illinois, USA were native or originated from commercially produced sources (Siegel et al. 2001). Native Bti was present at low levels in most of the habitats sampled except bromeliads and metal containers. Colonies of native Bti were present in 27% of temporary woodland pools sampled. Bs was not detected in untreated habitats. Commercially produced Bti and Bs applied to tyres was detected up to nine months after application.

## 5. Types of Bti formulations

**As stated in Glare and O'Callaghan (1998) there are several types of formulations of Bti including liquids, pellets and briquettes. Formulation can greatly influence environmental persistence and thus efficacy. There is no update for this section.**

## 6. Environmental safety of formulation components

**New information on the environmental safety of the formulation components of Bti products was not found in the literature.**

## 7. Host range

**Bti is known to be toxic to mosquitoes, blackflies and midges and may also be toxic to other less common groups of nematoceran dipterans such as the biting midges, Ceratopogonidae. Bti has been used to control mosquitoes, chironomid midges and blackflies throughout many regions of the world. Several new studies have been published since the original report by Glare and O'Callaghan (1998) and are discussed below.**

The abundance of chironomid larvae in mesocosms in a wetland in central Minnesota, USA was monitored after applications of Bti (Liber et al. 1998). Bti was applied as VectoBac® G to mesocosms twice at 21 day intervals. Five rates were applied (0.3X, 1X, 2.5X, 5X, 10X) where 1X was equal to 9 kg/ha, the rate used by the Minneapolis-St. Paul Metropolitan Mosquito Control District for early summer mosquito control. Chironomid numbers in Bti-treated mesocosms were compared to numbers in untreated control mesocosms. Chironomid larval populations were greatly reduced in the 10X treatment 4 days after the first Bti application. The

second application at 10X also reduced chironomid numbers, but the populations showed strong signs of recovery within 32 days.

Wahba et al. (1999) conducted laboratory studies with adult and immature stages of the sand fly, *Phlebotomus papatasi* to determine its susceptibility to Bti. Adults were fed a combination of Bti and either fructose or glucose while the immature stages were fed a larval diet containing Bti. The median lethal doses for adults fed the fructose and glucose mixtures were $1.3 \times 10^{-2}$ g/l and $3 \times 10^{-2}$ g/l, respectively. A 50% reduction in larvae occurred after exposure to $0.26 \times 10^{-5}$ g/l.

Laboratory toxicity tests were conducted to determine the susceptibility of third and fourth instars of two chironomid species, *Chironomus thummi thummi* (subfamily Chironominae) and *Psectrocladius psilopterus* (subfamily Orthocladiinae) (Yiallouros et al. 1999). Additionally, electron microscopic investigations were carried out to identify the damage caused to the gut by Bti in these species. Both species were susceptible to Bti with 24 hour LC50 estimates ranging from about 40- to 60-fold of the LC50 for *Stegomyia aegypti*. The LC50 for *C. thummi thummi* was 0.77 mg/l while the LC50 for *P. psilopterus* was 1.17 mg/l. Ultrastructural investigations of the midgut showed epithelium damage similar to that seen in mosquitoes.

### 7.1 LC50

The section on LC50 in Glare and O'Callaghan (1998) is still valid. One new study on this subject was published since the original report.

Laboratory toxicity tests were conducted to estimate the toxicity of a water-dispersible granule formulation of Bti (VectoBac WG; active ingredient: 3,000 Bti international toxic units (ITU)/mg) to third instars of six common Australian mosquito species, *Stegomyia aegypti*, *Ochlerotatus vigilax*, *Ochlerotatus notoscriptus*, *Culex sitiens*, *Culex annulirostris* and *Culex quinquefasciatus* (Russell et al. 2003). All of these mosquito species were extremely susceptible to the VectoBac WG formulation. The most sensitive species, *Cx. annulirostris* and *Cx. quinquefasciatus*, (LC95 value of 0.019 mg/l (ppm) were twice as susceptible as the most tolerant species, *Oc. notoscriptus*. (LC95 value of 0.037 ppm).

## 8. Effects on non-target organisms

**Bti is highly specific exhibiting toxicity to only a few select groups of flies. Therefore, most of the studies conducted on the effects of Bti on nontarget organisms have shown little or no effect. However, many new studies on Bti effects on nontarget organisms have been published since the report by Glare and O'Callaghan (1998) including a review article on this subject. The most important studies on nontarget effects are those by Hanowski et al. (1997), Hershey et al. (1998) and Niemi et al. (1999). These studies were conducted in wetlands in Minnesota, USA over several years and document that Bti applications have no effect on bird populations but greatly reduce aquatic insect biomass. However, it is unclear what the long-term consequences of insect reductions might mean to wetland health.**

The effects of Bti on target and nontarget organisms are reviewed by Boisvert and Boisvert (2000). Bti has been used in many countries on all continents since 1978 and numerous studies dealing with its effects on target and nontarget species have been published. Results of this review indicate that general predictions about the effects of Bti on nontarget organisms may be difficult to make due to differences in the species evaluated, differences in laboratory and field methodology employed, and the different Bti formulations used in various studies. Although Bti is usually thought of as a relatively specific toxicant that has little impact on nontarget organisms, certain nontarget organisms are negatively affected after either single or repeated applications. However, in organisms that don't have alkaline gut pH and thus the ability to dissolve the parasporal crystals, observed effects may be due to inert ingredients (formulation additives).

### *8.1 Invertebrates*

A long-term study on the effects of Bti and methoprene on the food web in freshwater mosquito habitat in Minnesota, USA was performed by Hershey et al. (1998) and also reported by Niemi et al. (1999). The first two years of the study involved intensive sampling prior to applications of the pesticides. This was followed by three years of treatments. Six applications of Bti were made each year at recommended label rates. During the first year of Bti application, minimal effects on nontarget organisms were detected. However, by the second and third year of pesticide application, major reductions in insect biomass occurred. Because Bti was probably only toxic to nematoceran Diptera, the observed effects were probably due to a disruption in the invertebrate food web. No negative effects on zooplankton or breeding birds could be attributed to pesticide treatment or reductions in insect biomass. The authors concluded that it was unclear what the long-term consequences of insect reductions might mean to wetland health.

Members of the mosquito (Culicidae), midge (Chironomidae), black fly (Simuliidae) and water flea (Cladocera) groups that inhabit alpine aquatic ecosystems are routinely exposed to Bti applied for control of mosquitoes (Rey et al. 1998). In this study, the susceptibility of the above-mentioned organisms to Bti was evaluated with laboratory bioassays. When Bti was applied at the concentration used for operational field application, all of the dipteran species were negatively affected, but Bti had no effect on the Cladocerans.

The toxicity of temephos, pirimiphos-methyl, methoprene and Bti to *Ochlerotatus vigilax*, an Australian saltmarsh mosquito vector of Ross River virus and to the nontarget shrimp species, *Leander tenuicornis*, was evaluated with laboratory studies in southeastern Queensland, Australia (Brown et al. 1999). Methoprene and Bti were the most selective products tested being much more toxic to *Oc. vigilax* than to *L. tenuicornis* with selectivity ratios (LC95 nontarget/LC95 target) of 255,000 and 38,000, respectively. Selectivity ratios for temephos and pirimiphos-methyl were 13 and 0.01 respectively indicating that these pesticides were much more likely kill nontarget species than methoprene or Bti. Field applications of methoprene and Bti did not negatively affect *L. tenuicornis*, but were highly effective in controlling *Oc. vigilax*. Temephos and pirimiphos-methyl were both effective against *Oc. vigilax*, but also killed 100% of caged *L. tenuicornis*.

The potential impact that mosquito control agents might have on nontarget chironomids was investigated by Laskowski et al. (1999). The effects of Bti, temephos, a carbohydrate gum

thickener (xanthan gum) and a modified starch (National 5370) on chironomid larvae in a wetland located in eastern Delaware, USA were measured. Only temephos had a negative impact on chironomids in this study.

The efficacy and nontarget effects of temephos, Bti and methoprene applied by helicopter to control mosquito larvae in mangrove swamps was determined on Sanibel Island, Florida, USA in May 1997 (Lawler et al. 1999). Three sites were treated with pesticides and three sites served as controls. Application rates for temephos (Abate) were 37 ml/ha (43% active ingredient (a.i.), Bti granules (Vectobac GTM) 5.606 kg/ha (200 International Toxic Units/mg), and methoprene (AltosidTM ALL), 213 ml/ha (5% a.i.). Caged *Ochlerotatus taeniorhynchus* were used to monitor efficacy while sentinel nontarget amphipods (Talitridae) were monitored for nontarget effects. Additionally, potential effects of pesticide applications on flying insects were assessed with light traps and by collecting dead insects that fell into tarps suspended under mangroves in areas treated with either temephos or methoprene. Each pesticide worked well in controlling mosquitoes but failures did occur occasionally. No amphipod mortality or mortality of flying insects was detected in the study sites.

The effects of granular Bti on aquatic nontarget invertebrates in Hong Kong were investigated by Dickman (2000). In 1998, pools along the Tai Tan River in the New Territories of Hong Kong were treated with Bti. The only organisms other than mosquitoes that appeared to be affected by the treatments were chironomid midges.

The effects of a combined formulation of Bti and liquid methoprene or sustained release methoprene pellets (Altosid® pellets) on nontarget organisms were studied in replicated saltmarsh ponds at maximum label rates (Lawler et al. 2000). Untreated ponds were used as controls. Caged *Ochlerotatus dorsalis* served as the target organism while the water boatmen, *Trichocorixa reticulata*, a predator, served as the nontarget species. Uncaged populations of invertebrates were also sampled with sweep nets. Both methoprene products and Bti killed caged mosquitoes. Altosid® pellets continued to kill *Oc. dorsalis* for 99 days. None of the pesticides affected survival or maturation of *T. reticulata,* or reduced abundance of uncaged invertebrates.

A study of the acute toxicity of seven pesticides used to control *Anopheles quadrimaculatus* around Craighead County, Arkansas, USA, to target and nontarget organisms was conducted in field and laboratory trials (Milam et al. 2000). The nontarget organisms evaluated in laboratory tests were the water fleas, *Ceriodaphnia dubia*, *Daphnia magna* and *D. pulex,* and the fat head minnow, *Pimephales promelas*. Additional tests were conducted with resident mosquito fish, *Gambusia affinis,* populations exposed to ditch-receiving waters. However, fat head minnows and *Gambusia* were not exposed to Bti in this study. Exposure to as much as 31.4 ug/l of the pesticides Dursban®, malathion, Permanone®, Abate®, Scourge®, Bti granular formulation and Bti liquid formulation, and Biomist® were required to effectively control *An. quadrimaculatus*. Substantial mortality of nontarget organisms occurred after exposure to concentrations as low as 2.7 ug/l for some of these pesticides. However, for the Bti granular formulation, the 24 hour acute LC50 was estimated at 626.6 mg/l for *D. magna*. The 48 hour LC50 estimates were 0.34 mg/l for *D. pulex* exposed to the Bti granular formulation, 3.90 ug/l for *D. pulex* exposed to Bti liquid, and 7.6 ug/l for the mosquito, *An. quadrimaculatus* exposed to the Bti liquid formulation. These results indicate that Bti granular formulation is much more toxic to *An. quadrimaculatus*

than to *Daphnia*.

The potential effects of Bti on nontarget invertebrates and fish after applications were made to control the black fly, *Simulium jenningsi* in the Susquehanna River, Pennsylvania, USA, were investigated by Jackson et al. (2002). Most of the invertebrates were unaffected by Bti including the chironomid midge, *Rheotanytarsus*, which was previously reported to be susceptible to Bti. Fish species composition and abundance in riffles did not change following the Bti application, indicating that Bti had no effect on fish.

A study was conducted to determine whether applications of Bti and temephos used to control the black fly, *Simulium pertinax* in two rivers in Brazil were having a negative impact on aquatic insects (deAraujo-Coutinho et al. 2003). Approximately 28,477 specimens of aquatic insects in the families, Hydropsychidae, Chironomidae, Bactidae, Simuliidae, Blephariceridae and Megapodagrionidae were collected during the study. Temephos reduced populations of Chironomidae and Simuliidae. However, Bti only affected Simuliidae.

Laboratory toxicity of Teknar HP-D, an improved biolarvicidal formulation of Bti, to the mosquito fish, *Gambusia affinis*, and two predaceous aquatic insects, the back swimmer, *Notonecta* sp. and the water bug, *Diplonychus indicus* was evaluated (Gunasekaran et al. 2004). Each species was exposed to concentrations equivalent to 1, 1.5 and 2 1itres/ha. Bti (Teknar HP-D) was not toxic to any of these species at the concentrations tested.

The effects of Bti, methoprene (Altosid®) and temephos (Abate®) on a biological control agent of the weed, purple loosestrife (*Lythrum alicaria*), the leaf feeding beetle, *Galerucella calmariensis*, was determined by Lowe and Hershberger (2004). Larvae and adult *G. calmariensis* were fed loosestrife foliage dipped in Abate® (375 g/l), Altosid® (250 g/l), and Bti (110 g/l). Eggs on cuttings of purple loosestrife were exposed by dipping the cuttings into solutions of the same concentrations used in the feeding study. However, pupae were exposed by emersion in solutions of Abate (474.4 ug/l) and Altosid (1,169.2 ug/l). None of the life stages of *G. calmariensis* were susceptible to Bti concentrations tested in this study. Only the egg and larval stages were susceptible to Altosid, but all life stages were susceptible to Abate.

### *8.2 Fish and amphibians*

In the original document by Glare and O'Callaghan 1998, there was no evidence in the scientific literature indicating that Bti negatively affected fish. New literature published since the original assessment also failed to find negative effects of Bti on fish species.

As mentioned in section 8.1, fish species composition and abundance in riffles did not change following the application of Bti for control of black flies in the Susquehanna River in Pennsylvania, USA (Jackson et al. 2002).

Acute toxicity studies of several insecticides including Bti were conducted on the crimson-spotted rainbowfish, *Melanotaenia duboulayi* in Australia (Brown et al. 2002). Bti had no acute toxic effect on this fish species even at 10 and 12.5 times estimated environmental concentration for a 15 cm deep body of water.

As discussed above in section 8.1, the study by Gunasekaran et al. (2004) showed that Bti had no negative effect on the mosquito fish, *Gambusia affinis*.

### *8.3 Mammalian toxicity*
No new studies on mammalian toxicity published after the report by Glare and O'Callaghan (1998) were found in the literature.

### *8.4 Birds (New Section)*
Long-term field studies with Bti used to control mosquitoes in wetlands in Minnesota, USA (discussed above) indicated that Bti has no effect on red wing black bird populations (Hershey et al. 1998, Niemi et al. 1999). A more detailed study on the effects of Bti on a bird community was conducted by Hanowski et al. (1997). Bti (Vectobac-G granules) had no effect on the bird community or on 19 individual bird species.

Measurements of bird populations in the nature protection area "Kuehkopf-Knoblochsaue" (2,369 ha) on the River Rhine in Hesse, central Germany, after a reconversion of cultivated fields to meadows was studied from 1994 to 1996 (Kreuziger 1998). Over a third of the species increased in abundance while less than 10% decreased. Most of the species showed changes in dispersion within the study area. However, bird species breeding in reed beds and along the water's edge decreased which may have resulted from reduced food-availability caused by Bti used to control mosquitoes in the River Rhine.

### *8.5 Algae (New section)*
Treatment of microcosms with VectoBac G (Bti) at the high dosage of 48.1 kg/ha, Bs water dispersible granules at 3.1 kg/ha, and Bti water dispersible granules at 0.6 kg/ha resulted in good control of immature mosquito populations but also suppressed the growth of two algal species, *Closterium* sp. and *Chlorella* sp. (Su and Mulla 1999b). The reduction in algal populations resulted in reduced photosynthesis, lower water turbidity and oxygen concentrations in the treatments compared to controls, especially during the hot season.

Boisvert and Boisvert (2000) in their review on Bti effects on nontarget organisms suggest that the declines in insect biomass observed by Hershey et al. (1998) and Niemi et al. (1999) may be explained by a reduction in algal biomass which in turn would have a major impact on the entire food web.

## 9. Persistence and activity in the environment

**Several new studies dealing with the persistence of Bti in the environment have been published since the report by Glare and O'Callaghan (1998).**

### *9.1 Persistence*
The section on persistence of Bti by Glare and O'Callaghan (1998) is very thorough. In general persistence of Bti is very short. Toxicity of Bti lasts only a few days at most and efficacy can be reduced within 24 hours. Several new studies have been published on this subject since the original report and are discussed below.

The persistence of Bti was tested over a five-month period in a low-temperature aquatic environment by placing diffusion chambers filled with a suspension of Bti (100 mg/l) and

different substrates (pond water, periphyton, sediments and vegetation), near the bottom of a large pond (Boisvert and Boisvert 1999). The chambers were removed at various time intervals to test toxicity to mosquito larvae. After one month the pond water substrate produced 50% of the initial toxicity, the periphyton substrate exhibited 30% of initial toxicity. The liquid phase of the vegetation substrate produced 30% of the initial toxicity for 84-154 days. The solid fraction of the vegetation remained toxic for up to five months.

The duration of action of Bti and Bs against larvae of *Anopheles stephensi* was evaluated in Russia (Ganushkina et al. 2000). Activity of each product was dependent on the initial concentration of spores. The optimal concentration of spores was determined to be at least 105 spores/ml for *An. stephensi* larvae. The number of larvae per test container also influenced efficacy and the duration of activity. The more larvae present in test vessels, the shorter the duration of kill. The presence of dead larvae in the test vessel also increased the duration of action by Bti as much as four times compared to test vessels where the dead larvae were removed.

The efficacy and persistence of several formulations of VectoBac® (Bti) were evaluated in 250-litre fibreglass containers (Vilarinhos and Monnerat 2004). The number of pupal *Stegomyia aegypti* in containers was used as a measure of control. Under sunlight conditions, control of *St. aegypti* dropped below 90% after just one week in containers treated with two concentrations of VectoBac CG (1 and 2 g/50 litres) and VectoBac Tablet (T) formulation at 1 tablet/100 litres. An increased concentration of VectoBac T (1 tablet/50 litres) provided 100% control for two weeks. VectoBac WDG (1 and 2 g/500 litres) provided 100% control for three weeks. Control of *St. aegypti* was generally longer in covered containers (without sunlight) in some cases lasting for up to nine weeks.

### *9.2 Effect of formulation on persistence*

The section on the effects of formulation on persistence by Glare and O'Callaghan (1998) covered the known literature and indicated that certain Bti formulations were more persistent than others. For example, briquette formulations are more persistent than liquid formulations. However, as a general rule, Bti products, even slow-release products, are not very persistent in the environment compared to other insecticides.

Field studies on the efficacy and persistence of a floating sustained release formulation of Bti applied at 15 kg/ha was evaluated against *Culex quinquefasciatus*, the vector of Bancroftian filariasis, breeding in cesspools and cesspits (Gunasekaran et al. 2002). Three applications of Bti were made and the study sites were evaluated for 179 days. Results indicated that the same level of recruitment (number of egg rafts laid) between control and treated sites occurred but that the abundance of *Cx. quinquefasciatus* larvae and pupae was significantly lower (>80%) in Bti treated habitats compared to controls.

Encapsulation of Bti and Bs with the protozoan, *Tetrahymena pyriformis infusoria* increased persistence of both products (Ganushkina et al. 2002). The bacteria appear to multiply in the digestive vacuoles of the protozoan.

The persistence of a Bti spore-toxin complex to UV light was increased by encapsulation with aluminum carboxymethylcellulose using green malachite, ponceau red and congo red (Ramirez-Lepe et al. 2003). Encapsulation increased stability under direct UV light but reduced toxicity.

The efficacy and persistence of a new formulation of Bti, Vectobac® DT (ABG-6499), a tablet formulation containing 3.4% of active ingredient (3,400 ITU/mg), was evaluated in Rome, Italy from June to September in 2002 for control of larval *Stegomyia albopicta* (Toma et al. 2003). Application of Vectobac DT to plastic buckets resulted in 100% control of *St. albopicta* within 24 hours of treatment. However, larvicidal activity only lasted for 48 hours thus requiring treatments to be performed every 8-10 days throughout the breeding season.

### *9.3 Application methods and rates*

The section on application rates and methods by Glare and O'Callaghan (1998) indicated that rates vary depending upon type of formulation, surface area of water to be treated, larval density and stage and water quality. These factors still determine the application rates used today. Several new studies on application rates and methods are presented below.

The efficacy of methylated soybean oil (MSO) alone, MSO and technical-grade Bti, Golden Bear Oil® (GB-1111) and a water-based Bti formulation was evaluated against third-fourth stage *Anopheles quadrimaculatus* larvae confined to sentinel cages in small rice plots (Dennett et al. 2000).The treatments were as follows: MSO with 2% Pyroter® added as a surfactant (MSO + PYR), MSO with 2% Pyroter and 4 g of Bti technical powder (MSO + PYR + Bti), GB-1111, a water-based formulation with 4 g of Bti technical powder (Bti + water), and untreated control. The most effective treatment was the Bti + water formulation which resulted in 71% control. MSO + PYR + Bti formulation achieved 64% control, while the MSO + PYR and GB-1111 produced 16 and 18% control, respectively, 24 hours after treatment. These results indicate that Bti is the most effective product of those tested for control of *An. quadrimaculatus.*

The influence of abiotic factors in streams on the performance and persistence of two commercial formulations of Bti (Teknar HP-D and Vectobac 1200L) was investigated by Boisvert et al. (2001a,b, 2002). They found that factors such as water temperature, stream discharge and the hyporheic zone (interstitial water between the streambed and groundwater) affected the behaviour, performance and loss of the residual dosage of Bti products applied to streams. Higher concentrations of Vectobac 1200L were recovered than Teknar HP-D and results indicated that the hyporheic zone produced a major loss of these products in the first metres of the stream. However, Teknar was more efficacious (less toxin were needed to kill 50% of black fly larvae) in cooler (16°C) and warmer water (19.5-22°C).

A study was conducted in Thailand to evaluate several approaches for the control of *Stegomyia aegypti* in water-storage containers (Mulla et al. 2004). VectoBac tablets (Bti 5%) and a new formulation of zeolite granules (ZG) of temephos (1%) were compared with the formulation presently used in Thailand, temephos SG (1%), by treating water-storage jars. Control of *St. aegypti* was achieved with VectoBac tablets at the dosage of 1 tablet or 0.37 g per 50 litres of water for 112 days. The two temephos formulations provided control for more than six months.

## 10. Gene transfer

**Although there is potential for transfer of genetic elements from Bti to other bacterial**

**species, and this has been demonstrated in the laboratory, there is no evidence that this has resulted in a dangerous new combination in nature (Glare and O'Callaghan 1998). This statement still holds true today.**

A search for organisms that could potentially be the recipients of gene transfer of Bti toxin genes was conducted by collecting cyanophytes from rice fields in malaria areas of Indonesia, China, French Guiana, Japan and south-east Turkey and in various *Anopheles* breeding places in Cameroon, Uganda and Syria (Cepak and Rettich 2001). Of the 35 genera and more than 60 species of cyanophytes identified, two unicellular strains, i.e., *Chroococcus* minor strain Cepak 1993/1 and *Synechococcus* sp. strain Cepak 1996/1 had the potenial for Bti gene introduction due to their easy ingestibility and digestibility and rapid growth in culture.

## 11. Resistance

**Concern over the possible development of resistance to Bti is warranted based on the fact that many insect species have developed resistance to various insecticides since the large-scale use of modern pesticides began. Some house fly populations and certain Lepidoptera (Glare and O'Callaghan 1998) have developed resistance to Bti. Low-level resistance or tolerance has been observed in some mosquito populations but a reversion back to susceptibility usually occurs very rapidly once a control programme ends. Several new studies have shown that mosquito strains resistant to Bs are usually very susceptible to Bti indicating that mosquito populations resistant to Bs can be controlled with Bti.**

The possible development of resistance to Bti in field populations of *Aedes vexans* in the Rhine area of Germany was investigated (Ludwig and Becker 1997). The susceptibility of larvae of *Ae. vexans* collected from the field in three untreated areas of Lake Constance and six Bti-treated areas from the Rhine Valley was assessed in the laboratory by comparing LC50 estimates and probit line slopes. No significant differences were detected among populations collected from untreated and Bti-treated areas.

To determine the effects of Bti applications on mosquito genetics, isozyme patterns of 13 field-collected populations of *Stegomyia aegypti* from Thailand before Bti application was initiated were compared to 10 populations collected after Bti treatment (Lerdthusnee and Chareonviriyaphap 1999). Results indicated that a genetic bottleneck occurred because populations collected after Bti treatment had lower genetic variability. However, recovery occurred in the following months probably due to immigration once the control programme was terminated.

Reports of Bs resistant strains of *Culex quinquefasciatus* developing tolerance to Bti led Poopathi et al. (1999b, 2000) to conduct resistance studies with this species in the laboratory. Populations of laboratory reared Bs-resistant *Cx. quinquefasciatus* were subjected to continuous selection pressure with a Bs 1593M strain for five years and tested for cross-resistance to Bti (IPS-82). Only a low level of tolerance to Bti was detected among the Bs selected resistant (GR) when compared to Bs unselected (GS) and normal (MS) strains of *Cx. quinquefasciatus*.

An Indian strain of *Culex quinquefasciatus* collected from Gandhinagar, Kochi, Kerala, in 1995 found to be highly resistant to Bs was very susceptible to Bti (Poopathi and Baskaran 2001).

*Culex quinquefasciatus* populations were exposed to Bs strains C3-41 and IAB59 in the laboratory for 13 and 18 generations where they attained 145,000- and 48.3-fold resistance, respectively, compared to a susceptible laboratory colony (SLCq) (Yuan et al. 2003). However, the Bs resistant mosquito populations remained highly susceptible to Bti. These results indicate that mosquito populations that become resistant to Bs can be controlled with Bti.

A review of mosquito control programmes aimed at reducing the incidence of malaria in India, indicated that resistance to Bs is becoming an increasing problem but mosquito populations have not shown significant development of resistance to Bti (Mittal 2003). However, the short persistence of Bti requires weekly application in most habitats. To overcome the problem of short environmental persistence, the development of new slow release formulations and genetically engineering biolarvicides by transplanting mosquitocidal toxin genes of Bti and Bs in other environmentally compatible organisms is being investigated by different scientists.

Zahiri and Mulla (2003) conducted laboratory studies to determine whether rotation of Bs and Bti or a mixture of both delayed or prevented the development of resistance in a colony of Bs-susceptible California *Culex quinquefasciatus*. Exposure to Bs alone for 15 generations resulted in resistance. Resistance to Bs developed more rapidly and was more severe when Bs and Bti were rotated. However, no resistance was detected in *Cx. quinquefasciatus* that had been exposed to the mixture of Bs and Bti for 36 generations. Based on these results the authors concluded that mixtures of Bs and Bti can be used to delay or prevent Bs resistance.

The larvae of *Culex pipiens* were repeatedly exposed to Bti in the laboratory to test for the development of resistance (Saleh et al. 2003). Only a 2.78-fold tolerance to Bti was induced in *Cx. pipiens* after 20 generations of selection. Reduced reproduction was observed in the tolerant population. Three generations after stopping the selection with Bti, tolerance of *Cx. pipiens* to Bti decreased by approximately 58%.

A study to detect the development of resistance of five field populations of *Anopheles sinensis* from southern and central China to Bti was conducted (Hongyu et al. 2004). Low levels of resistance were detected in some of the strains with 1.7 to 5.9-fold decreases in susceptibility compared to a naïve laboratory strain.

Four field strains of *Stegomyia albopicta* collected from 2002-2003 from Alabama and Florida, USA were evaluated for resistance to permethrin, deltamethrin, resmethrin, chlorpyrifos, malathion, propoxur, fipronil, imidacloprid, spinosad and Bti (Liu et al. 2004a). Although some of these strains showed a relatively low level of resistance to deltamethrin and chlorpyrifos, resistance to Bti was not detected.

Moderate to high levels of resistance and cross-resistance to several insecticides were detected in three strains of *Culex quinquefasciatus* in Alabama and Florida, USA (Liu et al. 2004b). Resistance ratios varied from 5 to 720 fold for permethrin, resmethrin, malathion, deltamethrin, chlorpyrifos, fipronil and imidacloprid. All three strains were highly susceptible to Bti and

spinosad, indicating that these products could be used to control resistant strains of *Cx. Quinquefasciatus.*

## 12. Host and environmental factors affecting efficacy

**The section on host and environmental factors affecting the efficacy of Bti by Glare and O'Callaghan (1998) is still valid today.**

### *12.1 Feeding behaviour of host*

The section on feeding behaviour of the host by Glare and O'Callaghan (1998) covers the differences in feeding behaviour among mosquito species which influences uptake of Bti. This section is still valid today and no new additional literature on this subject was found.

### *12.2 Inoculum and host density*

In the section on inoculum and host density in the original report Glare and O'Callaghan indicate that inoculum and host density play a role in the efficacy of Bti. This section is still valid and no new literature on this subject was found.

### *12.3 Developmental stage of larvae*

In this section, Glare and O'Callaghan (1998) show that the older the mosquito instar, the less susceptible it is to Bti. This is still a valid conclusion today. One new study on this subject was found and is presented below.

Studies on the effects of age on susceptibility of fourth instar *Chironomus tepperi* showed that younger fourth instars were more susceptible than older larvae (Stevens et al. 2004).

### *12.4 Other organisms*

The section is still current. Other organisms, particularly filter feeders can reduce the number of Bti cells available to control mosquitoes thus reducing efficacy. No new literature was found on this subject.

### *12.5 Water quality*

This section is still current. Water quality, including the presence of pollutants, salinity and inorganic material can affect the efficacy of Bti. No new literature was found on this subject.

### *12.6 Temperature*

Water temperature has been shown to have an effect on the toxicity of Bti; lower efficacy being seen at lower temperatures. This conclusion has not changed since the report by Glare and O'Callaghan (1998) and is probably a result of reduced feeding of target species. Three new studies on this subject are presented below.

The effects of temperature on the efficacy of Bs (Bs-IPS88) and Bti H14 (Bti-IPS82) to fourth instars of *Culiseta longiareolata* was evaluated under laboratory conditions (Katbeh-Bader et al. 1999). Larvae were found to be more susceptible to Bs than to Bti at 25 $\pm$ 1°C and mortality increased significantly for both products as temperature increased from 20 to 28°C.

The efficacy of VectoBac® TP (Bti) to the saltmarsh mosquito, *Ochlerotatus squamiger* at different temperatures was evaluated (Christiansen et al. 2004). An LD90 of 0.223 mg/l was obtained for third and early fourth instars exposed to Bti at 14°C, but this value more than doubled, meaning that Bti was less toxic, when larvae were exposed to Bti at 6°C. A field trial in Salinas, California, USA corroborated the laboratory results indicating that Bti efficacy is influenced by temperature and that lower temperatures reduce effectiveness.

Stevens et al. (2004) evaluated the effect of temperature on the Bt formulations, VectoBac WDG, and a spore/crystal mixture derived from VectoBac WDG strain and VectoLex WDG, a commercial Bs formulation (650ITU/mg) on the midge, *Chironomus tepperi*. As temperatures were reduced from 30 to 17.5$^{o}$C, six-fold declines in activity of VectoBac WDG and the spore/crystal mixture were observed.

### *12.7 Vegetative cover and food*

The presence of dense vegetation can reduce the efficacy of Bti (Glare and O'Callaghan 1998). Two new studies on this subject have been published since the original report.

Ye-ebiyo et al. (2003) found that maize pollen greatly increased the feeding of *Anopheles arabiensis* larvae which in turn enhanced feeding on Bti increasing the efficacy of this pesticide.

High populations of algae have been reported to reduce the efficacy of Bti in the USA (Stephens et al. 2004). A laboratory study was conducted to evaluate the effects of four genera of algae (*Microcytis, Scenedesmus, Dictyosphaerium* and *Chlorella*) commonly detected in Pennsylvania rivers where the effectiveness of Bti for control of black fly larvae has declined. Larvae of the black fly, *Simulium vittatum* cytospecies IS-7 were exposed to Bti in the presence of these algae. *Scenedesmus* was shown to significantly reduce Bti-induced mortality when present at concentrations of 16,000 cells/ml. The other species had no effect on the efficacy of Bti to *S. vittatum* even at concentrations as high as 250,000 cells/ml. These results indicate that only certain species of algae can affect the efficacy of Bti to black flies. However, no mechanism for this effect was determined in this study but the authors suggested that the size of certain algae species might interfere with black fly feeding.

### *12.8 pH*

This section is still current. Bti has been found to be relatively insensitive to the range of pH found in surface water (Glare and O'Callaghan 1998). No new literature was found on this subject.

### *12.9 UV*

This section is still current. Sunlight has been shown to reduce the efficacy of Bti. Three new studies were found dealing with the effects of light on Bti persistence.

Nayar et al. (1999) compared the efficacy of three formulations of Bti, a technical powder (VectoBac® TP, 5,000 international toxic units (ITU)/mg), an aqueous suspension (VectoBac® 12AS, 1,200 ITU/mg), and a granular formulation (VectoBac® CG, 200 ITU/mg) under laboratory conditions for efficacy against larvae *Ochlerotatus taeniorhynchus* and *Culex nigripalpus*. A significant reduction in toxicity was observed in VectoBac TP and VectoBac 12AS after exposure to high light intensity (140,000-170,000 lux).

The persistence of a Bti UV light was discussed in section 9.2. Encapsulation of Bti with aluminum carboxymethylcellulose increased stability under direct UV light (Ramirez-Lepe et al. 2003).

As discussed in section 9.1, Vilarinhos and Monnerat (2004) found that Bti persisted substantially longer in closed containers when compared to exposure to direct sunlight.

### *12.10 Bacteriophage*

This section is still current.

### *12.11 Efficacy comparisons*

Three papers on efficacy comparison are discussed in the original report by Glare and O'Callaghan (1998). Fifteen new papers on this subject have been published since the original report and are discussed below.

A comparison of the toxicity of a flowable formulation of Bti to *Stegomyia aegypti* was carried out in six quality control laboratories in four countries (Skovmand et al. 1998). Significant differences were found in the toxicity of Bti among the laboratories. These differences might be due to age, stage, and strain of larvae used, amount and type of food provided to larvae, and processing of samples.

Sustained release granular formulations of Bs strain 2362 and Bti that were locally produced were compared to commercial liquid concentrates of the same bacteria in cesspits and puddles in Ouagadougou, Burkina Faso (Skovmand and Sanogo 1999). A granular formulation of Bs applied at 30 kg/ha, reduced *Culex quinquefasciatus* 99% for at least 28 days in cesspits, whereas the same dosage of two Bti granular formulations and commercial liquid formulations of Bs and Bti gave 95% control for 8-14 days. Bti killed more mosquito species than Bs, being an effective control of *Cx. decens*, *Cx. cinereus* and psychodid larvae. Applications of granular and liquid Bs gave 60-97% control of *Anopheles gambiae* for 10 days in rain puddles.

A comparison of laboratory toxicity of five organophosphate insecticides (OPs), (chlorpyrifos, chlorpyrifos methyl, fenthion, malathion and temephos), three pyrethroids (bifenthrin, cypermethrin and permethrin), one phenyl pyrazole (fipronil), two microbial pesticides (Bti and Bs), and three insect growth regulators (IGRs) (diflubenzuron, methoprene and pyriproxyfen) to field-collected *Culex quinquefasciatus* larvae from urban Dhaka, Bangladesh was reported by (Ali et al. 1999). The ranking of toxicity from most toxic to least toxic was: fipronil> IGRs > pyrethroids > microbials > OPs.

Three formulations of Bti, a technical powder (VectoBac® TP, 5,000 international toxic units (ITU)/mg), an aqueous suspension (VectoBac® 12AS, 1,200 ITU/mg), and a granular formulation (VectoBac® CG, 200 ITU/mg) were tested in the laboratory under different biotic and abiotic conditions for efficacy against larvae of *Ochlerotatus taeniorhynchus* and *Culex nigripalpus* (Nayar et al. 1999). The larvae (second, third and fourth instars) of *Cx. nigripalpus* were 1.3-3-fold more susceptible to both VectoBac TP and VectoBac 12AS than were the respective instars of *Oc. taeniorhynchus*. The second instars of each species were several-fold more susceptible to these Bti preparations than were the fourth instars. Larvae under low density were more susceptible to Bti than larvae held in high densities in test cups. VectoBac TP and VectoBac 12AS were more toxic at 32-35°C than at 15-20°C. Increasing salinity from 0 (fresh water) to 50% sea water caused a gradual decline in efficacy of VectoBac TP and VectoBac 12AS to *Oc. taeniorhynchus* larvae.

Combinations of Bti (Vectobac 12AS®) and the chemical insecticides, Actellic 50EC®, Aqua Resigen®, Resigen® and Fendona SC®, were evaluated in East Malaysia for control of *Aedes* larvae and adults (Seleena et al. 1999). Nine different formulations were applied with a portable mist blower to single story half-brick houses. Larval and adult mortality were the recorded endpoints. The mixtures were significantly more effective than the chemical insecticides alone seven days after treatment except for the Actellic 50EC® and Vectobac 12AS® mixture. However, there was no significant difference in adult mortality when Bti was part of the mixture, indicating that the Bti formulation, Vectobac 12AS®, was not antagonistic to the adulticidal activity of the chemical insecticides.

Su and Mulla (1999a) compared new water-dispersible granule (WDG) formulations of Bti and Bs for control of *Culex* mosquitoes in microcosms. Control was achieved for 7-12 days with the minimum effective dosage for Bti WDGs with 4,000 International Toxic Units (ITU)/mg of 0.27-0.53 lb/acre. The minimum effective dosage for Bs WDGs with 350-630 ITU/mg was 0.05-0.10 lb/acre, which provided control for 14-20 days.

*Bacillus thuringiensis* serovar *medellin* strain 163-131 and *Bacillus thuringiensis* serovar *jegathesan* (Btjeg.) strain 367 are about 10 times less toxic to mosquito larvae than Bti but show promise as potential control agents for mosquitoes. Field studies to compare the efficacy of Bti to *Bacillus thuringiensis* serovar *medellin* strain 163-131 and Btjeg strain 367 for control of *Culex pipiens* (Montpellier strain) were conducted in Paris, France, and *Stegomyia aegypti* larvae (French Guiana strain) in Cayenne, French Guiana (Thiery et al. 1999). All three products exhibited similar efficacy against both mosquito species and under all conditions except water rich in organic matter. *Bacillus thuringiensis* serovar *medellin* had the lowest residual activity, both in the laboratory and in the field, but Btjeg remained toxic for as long as Bti.

A study of the efficacy of VectoLex® CG, a commercial corn-grit (CG) formulation of Bs serotypeH5a5b (strain 2362) and VectoBac® G a corncob granular (G) formulation of Bti as controls of *Culex quinquefasciatus* larvae was conducted in organically polluted roadside ditches and ponds in suburban Dhaka, Bangladesh (Ali et al. 2000). VectoBac G (11.2 kg/ha) provided 76% control of all instars of *Cx. quinquefasciatus* for three days but this level of control declined to 3% seven days after treatment. At a higher rate of application (22.4 kg/ha) 62-88% control of larvae for 7-10 days after treatment was achieved. VectoLex CG was more effective than

VectoBac. Applications of 5.6 kg/ha gave 67-97% larval reductions of third and fourth instar *Cx. quinquefasciatus* for at least 17 days after treatment. At a rate of 22.4 kg/ha, VectoLex completely controlled larvae for nearly five weeks.

The toxicity of three formulations of Bti (Teknar®, VectoBac® 12AS and Cybate®) to third instars of the arbovirus vectors, *Stegomyia aegypti*, *Ochlerotatus notoscriptus*, *Oc. vigilax*, and *Oc. camptorhynchus* was determined with laboratory studies in southeast Queensland, Australia (Brown et al. 2001). Lethal dose ratios, the LC50 of one species divided by another were used to compare susceptibility. *St. aegypti* and *Oc. notoscriptus*, were the least susceptible species to Bti while *Oc. vigilax* and *Oc. camptorhynchus* were the most susceptible. Cybate was less toxic than Teknar for *St. aegypti* and *Oc. notoscriptus*.

Boisvert et al. (2001c) developed a new method to evaluate the effects of Bti in streams and rivers. The method involves the use of gutters that are located on stream banks and has been shown to provide good reproducibility of mortality of the target pest(s) when recorded at various distances (stations) along the stream. Either single or several formulations can be tested repeatedly in the same portion of a stream and thus provide an accurate evaluation of the performance of a Bti formulation or a comparison among several products. The system is inexpensive and gives reliable statistical analysis.

The toxicity of two formulations of Bti H14 (Vectobac and BMP-1442X), Bs and insect growth regulators was evaluated against *Anopheles stephensi* and *Stegomyia aegypti*, vectors of malaria and dengue fever in India, respectively (Mittal et al. 2001). Both Bti formulations were found to be extremely toxic to both mosquito species, but Vectobac was the most toxic of the two. *St. aegypti* were more susceptible than *An. stephensi* to Bti. Bs was also very toxic to both species of mosquito.

The efficacy of the natural insecticide, spinosad was compared to temephos and Bti for the control of larval *Stegomyia aegypti* and *Anopheles albimanus* in laboratory and field studies (Bond et al. 2004). A 24-hour spinosad LC50 of 0.025 and 0.024 ppm was estimated for *St. aegypti* and *An. albimanus*, respectively. In a field trial in southern Mexico, eight weeks of control of *St. aegypti* was obtained with spinosad at 1 ppm and temephos (Abate®) 1% granules) applied at a rate of 100 $g/m^3$ water (the standard treatment rate). Applications of spinosad at 10 ppm prevented breeding for more than 22 weeks. In another field trial, spinosad at 5 ppm and temephos eliminated reproduction of *St. aegypti* for 13 weeks. Bti only provided control of *St. aegypti* for two weeks. Spinosad also controlled *Culex* mosquitoes and chironomids at the same rates mentioned above.

Cyclic use of Bti and Bs and combinations of these two products was evaluated in the laboratory to determine whether better control of *Culex quinquefasciatus* could be achieved (Gayathri et al. 2004). *Culex quinquefasciatus* was exposed to Bti and Bs at recommended field rates separately. The two products were also applied together at half the field rate and followed by a treatment with deltamethrin 2.8 EC when 50% of the mosquitoes had died. Results of this study indicated that the combination of both products followed by the deltamethrin application resulted in effective control of this species.

Zahiri et al. (2004) compared the toxicity of Bti, Bs (strain 2362) and the University of California Riverside (UCR) recombinant (producing toxins of both Bs and Bti) to larvae of *Culex quinquefasciatus* (susceptible and resistant to Bs 2362), and *Stegomyia aegypti*. Bti was highly toxic to both the susceptible and resistant strains of *Cx. quinquefasciatus* with LC50 values of 0.009 and 0.011 ppm and LC90 values of 0.057 and 0.026 ppm for Bs susceptible and resistant strains, respectively. Bti was also very toxic to *St. aegypti* with LC50 and LC90 values of 0.014 and 0.055 ppm, respectively. The UCR recombinant was equally toxic to the susceptible and resistant strains of *Cx. quinquefasciatus* with LC50 values of 0.005 and 0.009 and LC90 values of 0.030 and 0.043 ppm, respectively. There were basically no differences in the toxicity of Bti and the UCR recombinant to the susceptible and resistant strains of *Cx. quinquefasciatus*. The UCR recombinant was highly toxic to *St. aegypti* with LC50 and LC90 values of 0.023 and 0.064 ppm, respectively. The susceptible strain of *Cx. quinquefasciatus* was very susceptible to Bs with LC50 and LC90 values of 0.006 and 0.024 ppm, respectively. However, Bs was not very toxic to *St. aegypti* larvae. In additional studies, four experimental corn grit formulations of Bti (VBC 60021), Bs (VBC 60022), UCR recombinants VBC 60023 (7.89%), and VBC 60024 (1.87%) were evaluated in microcosms against Bs-susceptible *Culex* mosquitoes. Bti and the UCR recombinants showed similar activity initially providing high to moderate control for 2 to 7 days post-treatment. However, Bs and a high concentration of the UCR recombinant (VBC 60023) provided longer control (7-21 days) than the other two products.

*Anabaena* PCC7120 (A. 7120) a filamentous, heterocyst-forming cyanobacterium capable of nitrogen fixation was engineered with mosquitocidal toxin genes from Bti (Lluisma et al. 2001) to determine whether this modified organism had potential as a new product for control of mosquitoes. Results of this study showed that Bti genes were stable in A. 7120, continued to be mosquitocidal through four years of culture, and growth of the recombinant clones was comparable to the wild type under optimal growth conditions thus indicating that growth was not compromised by the expression of toxin genes.

## Use of Bti in Control and Eradication Programmes (New Section)

**Several mosquito control and eradication programmes have been conducted in various parts of the world using Bti. These programmes are summarised here.**

A study was conducted to evaluate the control efficacy of Bti and an indigenous larvivorous fish, *Aplocheilus blocki* on malaria transmitting mosquitoes in Goa, India in 1993 and 1994 (Kumar et al. 1998). Weekly application of Bti at a rate of 1 $g/m^2$ and introduction of the indigenous larvivorous fish in major breeding habitats of *Anopheles stephensi* resulted in significant declines in malaria incidence and populations of *An. stephensi*.

A field assessment of the effectiveness of larvicide and adulticide treatments against mosquitoes at a constructed wetland in San Jacinto, California, USA was made (Walton et al. 1998). Bti treatments (Bactimos® pellets) applied at a rate of 19 kg/ha did not appear to affect larval or emergent adult populations of *Culex* mosquitoes. However, two applications of Bs (Vectolex® CG) at a rate of either 19 or 23.6 kg/ha reduced populations to almost undetectable levels along with a large decline in adult emergence.

Small field plot evaluations were conducted targeting *Cx. annulirostris* with two Bti formulations, VectoBac WG and VectoBac 12 aqueous solution (AS) (a.i.): 1,200 Bti ITU/mg (Russell et al. 2003). Caged third instars were exposed weekly to six concentrations of the WG formulation ranging from 0.004-0.13 ppm and three concentrations of the 12AS formulation ranging from 0.04-0.13 ppm. Concentrations of 0.008 ppm VectoBac WG and 0.04 ppm VectoBac 12AS produced 96% larval control 48 hours after treatment, but no residual control was evident after one week.

Batra et al. (2000) conducted a study to evaluate the effectiveness of three different formulations of Bti for control of *Stegomyia aegypti* breeding in desert coolers and tyres. The Bti formulations evaluated were: VectoBac tablets, VectoBac granules and Bacticide powder, at the application rate of 0.75, 2, and 1 g per cooler, respectively, and VectoBac tablets at 0.75 and 0.375 g per tyre. Results of this study indicated that all three formulations provided 100% control of *St. aegypti* in both coolers and tyres for 2-3 weeks.

Furutani and Arita-Tsutsumi (2002) found that Bti provided excellent control of the Asian tiger mosquito, *Stegomyia albopicta* in non-circulating hydroponic tanks in Hawaii.

An integrated pest management programme in Thailand indicated that a combination of Bti and a copepod species, *Mesocyclops aspericornis* supplemented with rice grains could be used to effectively control larvae of *Stegomyia aegypti* in water containers (Kosiyachinda et al. 2003). The treatments evaluated in this study were: copepods only, Bti only, copepods and Bti together with the addition of a food supplement (rice grains) added to water containers containing set numbers of mosquito larvae. Eight weeks after treatment, Bti alone reduced *St. aegypti* populations by 90%, copepods alone reduced mosquito populations 67% and the combination of the two reduced populations 99.5%. Furthermore, the addition of the food supplement resulted in copepod populations that were three times higher than those without the supplement.

A comparison of the effectiveness of granular formulations of Bti serotype H14 (Vectobac® G, 200 ITU/mg) and Bs serotype H5a5b (Vectolex® CG, 670 Bs ITU/mg) and temephos for control of *Anopheles arabiensis* and other mosquitoes was made in breeding habitats in three sites, Gash-Barka, Anseba and Debub zones, in Eritrea (Shililu et al. 2003). The objectives of this study were to determine the optimal application rates and persistence of each product so that the most effective product(s) could be used to control malaria. Two rates of Bti (5.6 and 11.2 kg/ha) and Bs (11.2 and 22.4 kg/ha) were evaluated while temephos was applied at a rate of 100 ml/ha. All of these products caused high levels of mortality of the main malaria vector species, *An. arabiensis*, and other mosquito species (*An. cinereus*, *An. pretoriensis* and *Culex quinquefasciatus*). The activity of Bti and Bs varied depending on breeding habitat, mosquito species and ecology of the area. Bti and Bs exhibited similar persistence (2-3 week) and were as effective as temephos over a 2-3 week period. The *Bacillus* products were less effective where high levels of algae were present or in fast flowing streams primarily because of the inability to penetrate algal mats and the dilution effect, respectively. Results of this study showed that bimonthly applications of the two *Bacillus* products to streambed pools, rain pools and similar habitats should result in control of the anopheline mosquito populations.

Studies to determine the efficacy of a new water-dispersible granular (WDG) formulations of Bti (VectoBac®) and Bs (VectoLex®, Valent BioScience Corp., Illinois, USA) against larval *Anopheles gambiae* sensu lato mosquitoes were conducted in a malaria endemic area around Lake Victoria, Western Kenya (Fillinger et al. 2003). Initial laboratory studies indicated that *An. gambiae* was very susceptible to both products but that larvae were more susceptible to Bs than to Bti. Weekly field applications of 200 g/ha Bti WDG, representing the LC95 of the laboratory tests, completely suppressed emergence of mosquitoes. The Bs product was also very effective and had longer persistence in the field significantly reducing larvae for up to 11 days post-treatment at application doses of either 1 or 5 kg/ha. Conclusions of this study were that the main malaria vector in the study area is highly susceptible to these microbial control agents.

A study was conducted to determine the effectiveness of space applications of several insecticides for the control of malaria in Ranau, Malaysia (Seleena et al. 2004). Several villages were treated monthly for control of *Anopheles balabacensis baisas*. The number of cases of malaria and populations of *An. balabacensis baisas* were the endpoints evaluated. Some villages were treated with the adulticide, alpha cypermethrin (Fendona SCR/IOSCR) at 2 g a.i./10,000 $m^2$, others were treated with the larvicides, Bti (Vectobac 12ASR) at 500ml/10,000 $m^2$ or Bs (Vectolex WGR) at 500 g/10,000 $m^2$, and still other villages were treated with a mixture of adulticides and larvicides. An untreated village served as the control. Applications of alpha cypermethrin significantly reduced *An. balabacensis baisas* populations. Bti did not reduce *An. balabacensis baisas* populations in Pinawantai village but applications of Bs did reduce populations in this village. A significant reduction in malaria cases occurred in all of the treated villages indicating that space application of larvicides/adulticides or a mixture of both is able to reduce the malaria vector population and malaria transmission. The authors suggested that larger scale studies need to be undertaken to determine whether space application of insecticides together with other malaria control measures will be able to eradicate malaria.

## 13. Discussion and Conclusions

**Since the report of Glare and O'Callaghan (1998), many new studies on the effectiveness of Bti as a control for mosquitoes and the impact of Bti on nontarget organisms have been published. Bti continues to be one of the most important tools for control and eradication of mosquitoes throughout the world. Although development of resistance continues to be a concern, there are no examples of full-scale resistance to Bti being developed in wild mosquito populations. Only low-level resistance or tolerance has been observed. Several important studies on the impact of Bti on nontarget organisms in the field have been published since the report by Glare and O'Callaghan (1998). Long-term studies indicate that continued application of Bti to wetlands can greatly reduce the biomass of aquatic insects. This reduction however, did not result in a loss of ecosystem function. However, it is still unclear what the long-term consequences of large reductions in insect biomass might mean to wetland health. Based on the original report by Glare and O'Callaghan (1998) and an evaluation of new literature published after their report, Bti remains an effective tool for control and eradication of mosquitoes that poses little risk to human health and the environment.**

### *13.1 Is Bti safe for use in New Zealand?*

The conclusions by Glare and O'Callaghan (1998) are still valid. Bti appears to pose little hazard to human health and wildlife when used according to label instructions.

### *13.2 Risks to New Zealand fauna*

The conclusions by Glare and O'Callaghan (1998) are still valid. Bti affects relatively few groups of insects, the primary nontarget groups being Chironomidae, Simuliidae and non-pest Culicidae.

### *13.3 Other agents*

#### 13.3.1 Chemicals

A few new chemicals are being evaluated as potential controls for mosquitoes. Fipronil and spinosad are potential candidates that have been shown to be toxic to mosquitoes. However, more work needs to be done on large-scale field efficacy and effects on aquatic nontarget organisms. *Daphnia pulex* were found to be quite susceptible to spinosad, but spinosad was much less toxic than diazinon to this species (Stark and Vargas 2003). Fipronil was also found to be toxic to *D. pulex* but at the concentrations used for fruit fly control programmes in the South Pacific and Australia, it poses little risk to this species (Stark and Vargas in press).

#### 13.3.2 Insect growth regulators

Novaluron a new chiton synthesis inhibitor has been shown to be an effective control for *Stegomyia aegypti* in the laboratory and the field (Mulla et al. 2003). Emergence of adults was 100% after exposure of second and fourth instars to concentrations of 0.25 to 1.0 ug/l. Furthermore, Novaluron is a persistent control agent that has been shown to provide control of *St. aegypti* for 60 to 190 days depending on the concentration applied to water storage containers in Thailand.

#### 13.3.3 Microbial agents

##### 13.3.3.1 *Bacillus thuringiensis* strains other than Bti

In section 12.11 *Bacillus thuringiensis* serovar *medellin* strain 163-131 and *Bacillus thuringiensis* serovar *jegathesan* (Btjeg.) strain 367 were discussed. These products are approximately 10 times less toxic to mosquito larvae than Bti but show promise as potential control agents for mosquitoes (Thiery et al. 1999).

##### 13.3.3.2 *Bacillus sphaericus*

Bs has been discussed in several sections of this document, in particular in section 12.11 and in the new section entitled "Use of Bti in Control and Eradication Programmes". Bs is generally not as effective as Bti and development of resistance to Bs has become a problem in many areas of the world.

##### 13.3.3.3 *Lagenidium giganteum*

Hallom et al. (2000) compared the efficacy of LaginexTM, a biological larvicide containing *Lagenidium giganteum*, and VectobacTM-12AS (Bti) in plastic pools containing laboratory-

reared *Culex quinquefasciatus* larvae in Panama City, Florida, USA. LaginexTM controlled larvae for 20 days compared to 10-day control with Vectobac-12AS.

#### 13.3.3.4 *Romanomermis* spp.

This section of the original report is still valid.

#### 13.3.3.5 *Culicinomyces clavisporus*

This section of the original report is still valid.

#### 13.3.3.6. Other microbial agents

This section of the original report is still valid.

### *13.4 Efficacy of agents*

This section of the original report is still valid.

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## 15. References

**Ali, A., Chowdhury, M., Hossain, M.I., Mahmud-Ul-Ameen, Habiba, D.B., Aslam, A.F.M**. 1999. Laboratory evaluation of selected larvicides and insect growth regulators against field-collected *Culex quinquefasciatus* larvae from urban Dhaka, Bangladesh. *Journal of the American Mosquito Control Association* **15:** 43-47.

**Ali, A., Chowdhury, M.A., Aslam, A.F.M., Mahmud, A., Hossain, M.I., Habiba, D.B.** 2000. Field trials with *Bacillus sphaericus* and *Bacillus thuringiensis* serovar. *israelensis* commercial formulations against *Culex quinquefasciatus* larvae in suburban Dhaka, Bangladesh. *Medical Entomology and Zoology* **51:** 257-264.

**Batra, C.P., Mittal, P.K., Adak, T.** 2000. Control of *Aedes aegypti* breeding in desert coolers and tires by use of *Bacillus thuringiensis* var. *israelensis* formulation. *Journal of the American Mosquito Control Association* **16:** 321-323.

**Bond, J.G., Marina, C.F., Williams, T.** 2004. The naturally derived insecticide spinosad is highly toxic to *Aedes* and *Anopheles* mosquito larvae. *Medical and Veterinary Entomology* **18:** 50-56.

**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.

**Boisvert, M., Boisvert, J., Aubin, A.** 2001a. Factors affecting black fly larval mortality and carry of two formulations of *Bacillus thuringiensis* subsp. *israelensis* tested in the same stream during a 3-year experiment. *Biocontrol Science and Technology* **11:** 711-725.

**Boisvert, M., Boisvert, J., Aubin, A**. 2001b. Factors affecting residual dosages of two formulations of *Bacillus thuringiensis* subsp. *israelensis* tested in the same stream during a 3-year experiment. *Biocontrol Science and Technology* **11:** 727-744.

**Boisvert, M., Boisvert, J., Aubin, A.** 2001c. A new field procedure and method of analysis to evaluate the performance of *Bacillus thuringiensis* subsp. *israelensis* liquid formulations in streams and rivers. *Biocontrol Science and Technology* **11:** 261-271.

**Boisvert, M., Boisvert, J., Aubin, A.** 2002. Influence of stream profile and abiotic factors on the performance and residual dosages of two commercial formulations of *Bacillus thuringiensis* subsp. *israelensis* during a 2-year experiment. *Biocontrol Science and Technology* **12:** 19-33.

**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.

**Brown, M.D., Carter, J., Watson, T.M., Thomas, P., Santaguliana, G., Purdie, D.M., Kay, B.H.** 2001. Evaluation of liquid *Bacillus thuringiensis* var. *israelensis* products for control of Australian *Aedes* arbovirus vectors. *Journal of the American Mosquito Control Association* **17:** 8-12.

**Brown, M.D., Carter, J., Thomas, D., Purdie, D.M., Kay, B.H.** 2002. Pulse-exposure effects of selected insecticides to juvenile Australian crimson-spotted rainbowfish (*Melanotaenia duboulayi*). *Journal of Economic Entomology* **95:** 294-298.

**Cavados, C.F.G., Majerowicz, S., Chaves, J.Q., Araujo-Coutinho, C.J.P.C., Rabinovitch, L.** 2004. Pathological and ultrastructural effects of delta-endotoxins of *Bacillus thuringiensis* serovar *israelensis* in the midgut of *Simulium pertinax* larvae (Diptera, Simuliidae). *Memorias do Instituto Oswaldo Cruz* **99:** 493-498.

**Cepak, V., Rettich, F**. 2001. Screening of cyanophytes from mosquito breeding places (mostly rice fields) with emphasis on unicellular species - potential recipients of Bti genes. *Archiv fuer Hydrobiologie Supplement* **138**: 179-190.

**Christiansen, J.A., McAbee, R.D., Stanich, M.A., DeChant, P., Boronda, D., Cornel, A.J.** 2004. Influence of temperature and concentration of Vectobac® on control of the salt-marsh mosquito, *Ochlerotatus squamiger*, in Monterey County, California. *Journal of the American Mosquito Control Association* **20:** 165-170.

**deAraujo-Coutinho, C.J.P., Viviani-Cunha, A.B.P., Serra-Freire, N.M., de-Mello, R.P.** 2003. Evaluation of the impact of *Bacillus thuringiensis* serovar *israelensis* and temephos, used for the control of *Simulium* (*Chirostilbia) pertinax* Kollar, 1832 (Diptera, Simuliidae) on the associated entomofauna, Paraty, state of Rio de Janeiro, Brazil. *Memorias do Instituto Oswaldo Cruz* **98:** 697-702.

**Dennett, J.A., Lampman, R.L., Novak, R.J., Meisch, M.V.** 2000. Evaluation of methylated soy oil and water-based formulations of *Bacillus thuringiensis* var. *israelensis* and Golden Bear Oil® (GB-1111) against *Anopheles quadrimaculatus* larvae in small rice plots. *Journal of the American Mosquito Control Association* **16:** 342-345.

**Dickman, M.** 2000. Impacts of a mosquito selective pesticide, Bti, on the macroinvertebrates of a subtropical stream in Hong Kong. *Chemosphere* **41:** 209-217.

**Fillinger, U., Knols, B.G.J., Becker, N.** 2003. Efficacy and efficiency of new *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus* formulations against Afrotropical anophelines in Western Kenya. *Tropical Medicine and International Health* **8:** 37-47.

**Frampton, E.R.** 2004. Pathways of entry and spread of exotic mosquitoes, with particular reference to southern saltmarsh mosquito, *Ochlerotatus camptorhynchus*. *Report for New Zealand Ministry of Health*.

**Furutani, S.C., Arita-Tsutsumi, L.** 2002. Use of *Bacillus thuringiensis israelensis* and methoprene to control Asian tiger mosquito, *Aedes albopictus* (Skuse) (Diptera: Culicidae), in non-circulating hydroponic tanks. *Proceedings of the Hawaiian Entomological Society* **35:** 113-119.

**Ganushkina, L.A., Lebedeva, N.N., Azizbekyan, R.R., Sergiyev, V.P**. 2000. The duration of the larvicidal effects of sporocrystalline mass of the bacteria *Bacillus thuringiensis* spp. *israelensis* and *Bacillus sphaericus* in the laboratory setting. *Meditsinskaya Parazitologiya i Parazitarnye Bolezni* **4:** 25-29

**Ganushkina, L.A., Lebedeva, N.N., Azizbekyan, R.R., Yakubovich, V.Y., Sergiyev, V.P.** 2002. The duration of action of *Bacillus thuringiensis* spp. *israelensis* and *Bacillus sphaericus* after incapsulation with *Tetrahymena pyriformis infusoria*. *Meditsinskaya Parazitologiya I Parazitarnye Bolezni* **4:** 23-27.

**Gayathri, V., Jeyalakshmi, T., Shanmugasundaram, R., Murthy, P.B.** 2004. Rotational application of bioinsecticide with deltamethrin-an antilarval measure for the control of filarial vector, *Culex quinquefasciatus* (Culicidae: Diptera). *Journal of Environmental Biology* **25:** 419-421.

**Glare, T.R., O'Callaghan, M. 1998.** Environmental and health impacts of *Bacillus thuringiensis israelensis*. Report for the New Zealand Ministry of Health.

**Glare, T.R., O'Callaghan, M. 1999.** Environmental and health impacts of the insect juvenile hormone analogue, S-methoprene. Report for the New Zealand Ministry of Health.

**Gunasekaran, K., Prabakaran, G., Balaraman, K.** 2002. Efficacy of a floating sustained release formulation of *Bacillus thuringiensis* ssp. *israelensis* in controlling *Culex quinquefasciatus* larvae in polluted water habitats. *Acta Tropica* **83:** 241-247.

**Gunasekaran, K., Doss, P.S.B., Vaidyanathan, K.** 2004. Laboratory and field evaluation of Teknar HP-D, a biolarvicidal formulation of *Bacillus thuringiensis* ssp. *israelensis*, against mosquito vectors. *Acta Tropica* **92:** 109-118.

**Hallom, C.F., Schreiber, E.T., Vo, T., Bloomquist, M.A.** 2000. Field trials of three concentrations of LaginexTM as Biological Larvicide compared to VectobacTM-12AS as a biocontrol agent for *Culex quinquefasciatus. Journal of the American Mosquito Control Association* **16:** 5-8.

**Hanowski, J.M., Niemi, G.J., Lima, A.R., Regal, R.R.** 1997. Response of breeding birds to mosquito control treatments of wetlands. *Wetlands* **17:** 485-492.

**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.

**Hongyu, Z., Changju, Y., Jingye, H., Lin, 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.

**Kala, M,K., Gunasekaran, K.** 1999. Effect of *Bacillus thuringiensis* ssp. *israelensis* on the development of *Plasmodium gallinaceum* in *Aedes aegypti* (Diptera; Culicidae). *Annals of Tropical Medicine and Parasitology* **93:** 89-95.

**Katbeh-Bader, A., Khyami-Horani, H., Mohsen, Z.H.** 1999. Effect of temperature on the susceptibility of *Culiseta longiareolata* (Macquart) (Dipt., Culicidae) to two standard strains of biocontrol bacteria. *Journal of Applied Entomology* **123**: 629-631**.**

**Kosiyachinda, P., Bhumiratana, A., Kittayapong, P**. 2003. Enhancement of the efficacy of a combination of *Mesocyclops aspericornis* and *Bacillus thuringiensis* var. *israelensi*s by community-based products in controlling *Aedes aegypti* larvae in Thailand. *American Journal of Tropical Medicine and Hygiene* **69:** 206-212.

**Kreuziger, J.** 1998. Effects of large-scale restoration processes on the bird community of a river floodplain. *Vogelwelt* **119:** 65-90.

**Kumar, A., Sharma, V.P., Sumodan, P.K., Thavaselvam, D.** 1998. Field trials of biolarvicide *Bacillus thuringiensis* var. i*sraelensis* strain 164 and the larvivorous fish *Aplocheilus blocki* against *Anopheles stephensi* for malaria control in Goa, India. *Journal of the American Mosquito Control Association* **14:** 457-462.

**Laskowski, H.P., Larsen, A.C., O'Shea, G.F., Pittendrigh, B.R.** 1999. Effect of xanthan gum and traditional mosquito larvicides on chironomid larvae. *Wildlife Society Bulletin* **27:** 741-745.

**Lawler, S.P., Jensen, T., Dritz, D.A., Wichterman, G.** 1999. Field efficacy and nontarget effects of the mosquito larvicides temephos, methoprene, and *Bacillus thuringiensis* var. *israelensis* in Florida mangrove swamps. *Journal of the American Mosquito Control Association* **15:** 446-452.

**Lawler, S.P., Dritz, D.A., Jensen, T.** 2000. Effects of sustained-release methoprene and a combined formulation of liquid methoprene and *Bacillus thuringiensis israelensis* on insects in salt marshes. *Archives of Environmental Contamination and Toxicology* **39:** 177-182.

**Lerdthusnee, K., Chareonviriyaphap, T.** 1999. Comparison of isozyme patterns of *Aedes aegypti* populations collected from pre- and post-*Bacillus thuringiensis israelensis* treatment sites in Thailand. *Journal of the American Mosquito Control Association* **15:** 48-52.

**Liber, K., Schmude, K.L., Rau, D.M.** 1998. Toxicity of *Bacillus thuringiensis* var. *israelensis* to chironomids in pond mesocosms. *Ecotoxicology* **7:** 343-354.

**Liu, H., Cupp, E.W., Guo, A., Liu, N.** 2004a. Insecticide resistance in Alabama and Florida mosquito strains of *Aedes albopictus*. *Journal of Medical Entomology* **41:** 946-952.

**Liu, H., Cupp, E.W., Micher, K.M., Guo, A., Liu, N.** 2004b. Insecticide resistance and cross-resistance in Alabama and Florida strains of *Culex quinquefaciatus*. *Journal of Medical Entomology* **41:** 408-413.

**Lluisma, A.O., Karmacharya, N., Zarka, A., Ben-Dov, E., Zaritsky, A., Boussiba, S**. 2001. Suitability of Anabaena PCC7120 expressing mosquitocidal toxin genes from *Bacillus thuringiensis* subsp. *israelensis* for biotechnological application. *Applied Microbiology and Biotechnology* **57:** 161-166.

**Lowe, T.P., Hershberger, T.D.** 2004. Susceptibility of the leaf-eating beetle, *Galerucella calmariensis*, a biological control agent for purple loosestrife (*Lythrum salcaria*), to three mosquito control larvicides. *Environmental Toxicology and Chemistry* **23:** 1662-1671.

**Ludwig, M., Becker, N.** 1997. Studies on possible resistance against *Bacillus thuringiensis israelensis* in *Aedes vexans* (Diptera, Culicinae) after 15 years of application. *Zoologische Beitraege* **38:** 167-174.

**Milam, C.D., Farris, J.L., Wilhide, J.D.** 2000. Evaluating mosquito control pesticides for effect on target and nontarget organisms. *Archives of Environmental Contamination and Toxicology* **39:** 324-328.

**Mittal, P.K., Adak, T., Batra, C.P.** 2001. Comparative toxicity of selected larvicidal formulations against *Anopheles stephensi* Liston and *Aedes aegypti* Linn. *Journal of Communicable Diseases* **33:** 116-120.

**Mittal, P.K**. 2003. Biolarvicides in vector control: challenges and prospects. *Journal of Vector Borne Diseases* **40:** 20-32.

**Mulla, M.S., Thavara, U., Tawatsin, A., Chompoosri, J., Zaim, M. Su, T.** 2003. Laboratory and field evaluation of novaluron, a new acylurea insect growth regulator, against *Aedes aegypti* (Diptera: Culicidae). Journal of Vector Ecology **28:** 241-254.

**Mulla, M.S., Thavara, U., Tawatsin, A., Chompoosri, J.** 2004. Procedures for the evaluation of field efficacy of slow-release formulations of larvicides against *Aedes aegypti* in water-storage containers. *Journal of the American Mosquito Control Association* **20:** 64-73

**Nayar, J.K., Knight, J.W., Ali, A., Carlson, D.B., O'Bryan, P.D.** 1999. Laboratory evaluation of biotic and abiotic factors that may influence larvicidal activity of *Bacillus thuringiensis* serovar. *israelensis* against two Florida mosquito species. *Journal of the American Mosquito Control Association* **15:** 32-42.

**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.

**Poopathi, S., Mani, T.R., Rao, D.R., Baskaran, G., Kabilan, L.** 1999a. Effect of *Bacillus sphaericus* and *Bacillus thuringiensis* var. *israelensis* on the ultrastructural changes in the midgut of *Culex quinquefasciatus* Say (Diptera: Culicidae). *Journal of Entomological Research* **23:** 347-357.

**Poopathi, S., Mani, T.R., Rao, D.R., Baskaran, G., Kabilan, L.** 1999b. Cross-resistance to *Bacillus sphaericus* strains in *Culex quinquefasciatus* resistant to *B. sphaericus* 1593M. *Southeast Asian Journal of Tropical Medicine and Public Health* **30:** 477-481.

**Poopathi, S., Kabilan, L., Mani, T.R., Rao, D.R., Baskaran, G.** 2000. Observation of low tolerance to *Bacillus thuringiensis* var *israelensis* in *Culex quinquefasciatus* resistant to *Bacillus sphaericus. Entomon* **25:** 201-208.

**Poopathi, S., Baskaran, G.** 2001. Efficacy of *Bacillus thuringiensis* serovar *israelensis* against *Bacillus sphaericus* resistant and susceptible larvae of *Culex quinquefasciatus* Say. *Journal of Biological Control* **15:** 85-92.

**Ramirez-Lepe, M., Aguilar, O., Ramirez-Suero, M., Ecudero, B.** 2003. Protection of the spore-toxin complex of *Bacillus thuringiensis* serovar *israelensis* from ultraviolet irradiation with aluminum-CMC encapsulation and photoprotectors. *Southwestern Entomologist* **28:** 137-143.

**Rey, D., Long, A., Pautou, M.P., Meyran, J.C.** 1998. Comparative histopathology of some Diptera and Crustacea of aquatic alpine ecosystems, after treatment with *Bacillus thuringiensis* var. *israelensis*. *Entomologia Experimentalis et Applicata* **88:** 255-263.

**Russell, T.L., Brown, M.D., Purdie, D.M., Ryan, P.A., Kay, B.H.** 2003. Efficacy of VectoBac (*Bacillus thuringiensis* variety *israelensis*) formulations for mosquito control in Australia. *Journal of Economic Entomology* **96:** 1786-1791.

**Saleh, M.S., El-Meniawi, F.A., Kelada, N.L., Zahran, H.M.** 2003. Resistance development in mosquito larvae *Culex pipiens* to the bacterial agent *Bacillus thuringiensis* var. *israelensis. Journal of Applied Entomology* **127:** 29-32.

**Seleena, P., Lee, H.L., Chiang, Y.F.** 1999. Compatibility of *Bacillus thuringiensis* serovar *israelensis* and chemical insecticides for the control of *Aedes* mosquitoes. *Journal of Vector Ecology* **24:** 216-223.

**Seleena, P., Lee, H.L., Chooi, K.H., Junaidih, S**. 2004. Space spraying of bacterial and chemical insecticides against *Anopheles balabacensis baisas* for the control of malaria in Sabah, East Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health* **35:** 68-78.

**Shililu, J.I., G/M, T., Brantly, E., Githure, J.I., Mbogo, C.M., Beier, J.C., Fusco, R., Novak, R.J.** 2003. Efficacy of *Bacillus thuringiensis israelensis, Bacillus sphaericus* and temephos for managing *Anopheles* larvae in Eritrea. *Journal of the American Mosquito Control Association* **19:** 251-258.

**Siegel, J.P., Smith, A.R., Novak, R.J**. 2001. Recovery of commercially produced *Bacillus thuringiensi*s var. *israelensis* and *Bacillus sphaericus* from tires and prevalence of bacilli in artificial and natural containers. *Journal of the American Mosquito Control Association* **17:** 33-41.

**Skovmand, O., Thiery, I., Benzon, G.L., Sinegre, G., Monteny, N., Becker, N.** 1998. Potency of products based on *Bacillus thuringiensis* var. *israelensis*: Interlaboratory variations. *Journal of the American Mosquito Control Association* **14:** 298-304.

**Skovmand, O., Sanogo, E.** 1999. Experimental formulations of *Bacillus sphaericus* and *B. thuringiensis israelensis* against *Culex quinquefasciatus* and *Anopheles gambiae* (Diptera: Culicidae) in Burkina Faso. *Journal of Medical Entomology* **36:** 62-67.

**Stark, J.D., Vargas, R.I.** 2003. Demographic changes in *Daphnia pulex* (leydig) after exposure to the insecticides spinosad and diazinon. *Ecotoxicology and Environmental Safety* **56:** 334-338.

**Stark J.D., Vargas, R.I.** in press. Toxicity and hazard assessment of Fipronil to *Daphnia pulex*. *Ecotoxicology and Environmental Safety*.

**Stephens, M.S., Overmyer, J.P., Gray, E.W., Noblet, R.** 2004. Effects of algae on the efficacy of *Bacillus thuringiensis* var. *israelensis* against larval black flies. *Journal of the American Mosquito Control Association* **20:** 171-175.

**Stevens, M.M., Akhurst, R.J., Clifton, M.A., Hughes, P.A.** 2004. Factors affecting the toxicity of *Bacillus thurhigiensis* var. *israelensis* and *Bacillus sphaericus* to fourth instar larvae of *Chironomus tepperi* (Diptera:Chironomidae). *Journal of Invertebrate Pathology* **86:** 104-110.

**Su, T., Mulla, M.S**. 1999a. Field evaluation of new water-dispersible granular formulations of *Bacillus thuringiensis* ssp. *israelensis* and *Bacillus sphaericus* against *Culex* mosquitoes in microcosms. *Journal of the American Mosquito Control Association* **15:** 356-365.

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

**Thiery, I., Fouque, F., Gaven, B. Lagneau, C.** 1999. Residual activity of *Bacillus thuringiensis* serovars *medellin* and *jegathesan* on *Culex pipiens* and *Aedes aegypti* larvae. *Journal of the American Mosquito Control Association* **15:** 371-379.

**Toma, L., Severini, F., Bella, A., Romi, R.** 2003. A semifield evaluation of Vectobac® DT (ABG-6499), a new formulation of *Bacillus thuringiensis israelensis* for control of *Aedes albopictus*. *Journal of the American Mosquito Control Association* **19:** 424-429.

**Vilarinhos, P.T.R., Monnerat, R.** 2004. Larvicidal persistence of formulations of *Bacillus thuringiensis* var. *israelensis* to control larval *Aedes aegypti*. *Journal of the American Mosquito Control Association* **20:** 311-314.

**Wahba, M.M., Labib, I.M., El-Hamshary, E.M.** 1999. *Bacillus thuringiensis* var. *israelensis* as a microbial control agent against adult and immature stages of the sandfly, *Phlebotomus papatasi* under laboratory conditions. *Journal of the Egyptian Society of Parasitology* **29:** 587-597.

**Walton, W.E., Workman, P.D., Randall, L.A., Jiannino, J.A., Offill, Y.A.** 1998. Effectiveness of control measures against mosquitoes at a constructed wetland in Southern California. *Journal of Vector Ecology* **23:** 149-160.

**Ye-ebiyo,-Y., Pollack, R.J., Kiszewski, A., Spielman, A.** 2003. A component of maize pollen that stimulates larval mosquitoes (Diptera: Culicidae) to feed and increases toxicity of microbial larvicides. *Journal of Medical Entomology* **40:** 860-864.

**Yiallouros, M., Storch, V., Becker, N.** 1999. Impact of *Bacillus thuringiensis* var. *israelensis* on larvae of *Chironomus thummi thummi* and *Psectrocladius psilopterus* (Diptera: Chironomidae). *Journal of Invertebrate Pathology* **74:** 39-47.

**Yuan, Z.M., Pei, G.F., Regis, L., Nielsen-LeRoux, C., Cai, Q.X.** 2003. Cross-resistance between strains of *Bacillus sphaericus* but not *B. thuringiensis israelensis* in colonies of the mosquito *Culex quinquefasciatus*. *Medical and Veterinary Entomology* **17:** 251-256.

**Zahiri, N.S., Mulla, M.S.** 2003. Susceptibility profile of *Culex quinquefasciatus* (Diptera: Culicidae) *to Bacillus sphaericus* on selection with rotation and mixture of *B. sphaericus* and *B. thuringiensis israelensis*. *Journal of Medical Entomology* **40:** 672-677.

**Zahiri, N.S., Federic, B.A., Mulla, M.S.** 2004. Laboratory and simulated field evaluation of a new recombinant of *Bacillus thuringiensis* ssp. *israelensis* and *Bacillus sphaericus* against *Culex* mosquito larvae (Diptera: Culicidae). *Journal of Medical Entomology* **41:** 423-429.