Bactimos (Bti) WG Biological Larvicide Applications for Control of Non-Biting Midges

Frequently Asked Questions

1) **What is Bti?**
    a. Bti (*Bacillus thuringiensis* sub-species *israelensis*) is a naturally occurring bacteria found in the soils worldwide. It is a bacterial insecticide, not a chemical. In addition, Bti is different than any other type of bacteria in the world. It produces crystal shaped proteins that only kills the larval stage of mosquitoes, black flies (this specific biting fly is sometimes called a "buffalo gnat"), select non-biting midges, and other closely related flies.
        i. Latin Meaning: "*Bacillus*" means that this is a bacteria and "*thuringiensis*" relates to the province of Thuringia where a German scientist actually named this type/family of bacteria. "*israelensis*" is the name of the actual species of Bt, and there are dozens of different Bt types. This specific organism, *Bt israelensis* was actually found infecting mosquitoes in a puddle after a rainstorm in an Israeli desert.
    b. Bti is produced through fermentation, similar to making spirits or yogurt. The bacteria are fed nutrients and grow in large stainless steel tanks. After several days, nutrients are cut off. The bacteria die, leaving dead cells, crystal proteins and spores in the broth. The broth from these tanks is collected and then processed to make the various formulations of Bti that we produce.

2) **How does Bti work?**
    a. Bti produces a protein crystal during fermentation. These microscopic crystals are ingested by midge larvae as they feed. Select non-biting midges have an alkaline digestive system that allows for the crystals to dissolve and are then converted by specific enzymes into protein molecules that bind with unique receptor sites on the midge gut wall. This binding causes changes which destroy the walls of the midge larvae's gut. The larvae quit feeding within hours and quickly die.
    b. Bti affects only a select insect type (mosquitoes, select non-biting midges and other closely related flies in the sub-order of Diptera known as Nematocera); it must be ingested to work (hence it only works against larvae that are actively feeding), and it only sets up a reaction in their alkaline gut. It doesn't impact beneficial insect populations, as Bti works a bit like a 'lock and key' in the gut of susceptible insects. Other insects don't have the same structure and physiology for Bti to work on them.

3) **Is Bti safe?**
    a. Bti has undergone nearly 40 years of lab/field research and over 30 years of large-scale operational use in a variety of public health and nuisance programs around the globe. Bti is very target-specific with activity largely restricted to mosquitoes and related flies (in the sub-order Nematocera of the order Diptera).
    b. In addition to Bti's non toxicity to beneficial insects, numerous toxicology studies have shown Bti to be non-pathogenic and non-toxic to other forms of wildlife (birds, fish, mammals, etc.) and humans. The World Health Organization has approved the use of Bti for drinking water. This is particularly important in many regions around the globe that depend on potable water or rain water stored in vessels in and around their home. Unfortunately, these vessels are often prime locations for mosquito vectors of Zika virus to lay their eggs. The same species of mosquito also transmits the diseases dengue, chikungunya and yellow fever.

4) **What is Bactimos?**
    a. Bactimos is the brand name for a specific strain of Bti (strain AM65-52). All Bti strains can be 'lumped' into a category known as H14, but there are several strains within this category, and while all Bti products share some common characteristics, there are

significant differences amongst the strains of Bti that are used to produce the final products.

b. Strain is important to assure the bacteria's capacity to produce only the right proteins and express those proteins properly during fermentation, in fact Valent BioSciences' strain AM65-52 is only 1 of 2 strains evaluated and recommended by the World Health Organization as a mosquito larvicide in its public health pesticide evaluation program.
c. While genetics plays a crucial role, the environment in which bacterium grows can also have a significant impact on performance. While many traits may be similar, different environments can result in distinctly different individuals. The same holds true for the same species of bacteria being produced by two different manufacturers/environments. Bactimos' unique strain number for this bacterial non-biting midge larvicide is a critical link to product performance and quality expectations. The published literature shows that identical strains produced under different conditions can affect performance similar to different yoghurt cultures tasting different.
d. In addition, quality control (QC) requirements applied for fermentation of Bti strain AM65-52 are critical to ensure consistency and safety of Bactimos branded larvicides by ensuring that other potentially harmful pathogens are not produced during fermentation. The same QC measures are not utilized by all manufacturers of bacterial-based larvicides. As such, it is critical to differentiate these products based on the manufacturer's unique strain number. The use of generic strain identifers does not guarantee identical product performance or environmental safety.

5) What is Bactimos WG?

a. Bactimos WG Biological Larvicide is a Water Dispersible Granule formulation of Bti, strain AM65-52 for control of select non-biting midge larvae. It is designed for use with optimized spray application equipment that targets midge larval habitat (substrate/soil at bottom of water body).
b. The active ingredient behind Bactimos WG (Bti strain AM65-52) was the first bacterial larvicide to complete the World Health Organization (WHO) pesticide evaluation scheme.
c. Bactimos WG consists only of Bti strain AM65-52 and food-grade ingredients; it is gluten-free, egg-free and nut-free. It is approved by the Organic Materials Review Institute [OMRI] and the US Department of Agriculture for acceptance in the USDA National Organic Program [NOP].

6) **What are non-biting midges?**

a. Non-biting midges are the common term used for Chironomid midges (Diptera: Chironomidae). Worldwide, chironomids are the most widely distributed and frequently the most abundant aquatic macroinvertebrate in freshwater aquatic environments. Some species are also found in brackish and marine water and in terrestrial biotypes. In lentic freshwater eutropic urban environments, the benthic larval stages often occur in huge assemblages and upon emergence, adult midges can be a problem to the general public and industry, even though they do not bite or transmit diseases.
b. Chironomid midges have four distinct life stages: egg, larva, pupa and adult. The larval stage develops through 4 instars in less than two weeks depending on species and conditions. As with most insects, temperature and food availability control the length of time required for completion of their life-cycle.

Life Stage	**Description**
Eggs	Eggs are laid in mass on water or on firm, fixed objects along water edge.
Larvae	Hatched larvae develop in bottom of organic substrates where they feed upon algae, woody debris, detritus, macrophytes and invertebrates
Pupae	Pupae rise to the water surface and emerge as adults
Adults	Adults will live approximately 1 week depending upon the species and various biotic and abiotic factors. Males form swarms where they capture females for mating.

7) **How can you tell midges apart from mosquitoes**

Typical Mosquito	Chironomid Midge
Uses its proboscis to bite.	Cannot bite.
Wings are longer than its body.	Wings are shorter than its body
Larvae develop in water	Larvae develop in mud and detritus on the bottom of lakes and ponds
May carry disease	Do not carry diseases

8) **How susceptible are midges to Bactimos WG?**
   a. Most nuisance midges are in the sub-families Chironominae, Orthocladiinae and Tanypodinae. These classifications are an important aspect of any prospective suppression program as studies have demonstrated a wide range of susceptibility to Bti for the various sub-families. Typically species associated with the sub-family Chironominae are most susceptible to Bti while those associated with the Tanypodinae group are the least susceptible **(Ali 1996).** The filter feeding Chironominae are more likely to ingest Bti than the scrapers, shredders, collector-gatherers or predators (Tanypodinae, Orthocladids).
   b. **Boisvert & Boisvert (2000)** reported that chironomid larvae are typically 15-75 times less sensitive than some mosquito larvae to Bti; consequently, much higher rates are required to manage chironomids. It is assumed that the near neutral gut pH in many chironomid larvae is the reason for the lower susceptibility of chironomid larvae to Bti toxic proteins than other nematoceran Diptera, specifically mosquitoes. **Frouz et al. (2007)** showed that in two pestiferous Florida chironomid species, gut pH's of 6.7 – 7.6 were found compared to up to a pH of 11 for mosquitoes. Alkaline pH conditions are usually necessary for activation of spores and the solubilization of Bti toxic protein crystals.
   c.

9) **Why control midges?**
   a. The emergence and swarming of adult midges from aquatic habitats can create both nuisance and economic problems **(Armitage et al 1995):**
      i. Midges congregate in shady cool areas and deposit meconium or release egg-masses which stain surfaces. Adults can deface automobiles, walls, ceilings, curtains and other furnishings.
      ii. They are attracted to light which can cause great discomfort in residential areas.
      iii. Midges can be a problem for paint, paper, pharmaceutical and food processing industries where adults may contaminate final products.
      iv. Economic loss to the hotel and tourism industry can be affected by nuisance swarms that may create stressful conditions for tourists **(Anon., 1977).**
      v. Accumulation of dead adults and unattractive spider webs spun around resting adults requires frequent cleaning of properties.
      vi. Large numbers of dead midges can smell like rotting fish that persists in damp weather for several days after removal of adults.
      vii. Midges have been documented to cause allergic reactions including conjunctivitis, dermatitis, rhinitis and asthma.

10) **Should I be concerned and what should I do if a Bactimos WG program is conducted in my neighborhood?**
   a. There is nothing harmful or toxic in Bactimos WG; it is gluten-free, egg-free, and nut-free. It won't affect you, your family or your pets. If you have any medical issues, we would suggest that you consult your medical doctor and if you are still concerned or feel nervous for any reason, stay inside during the short time that your property/neighborhood is being treated.

b. Since applications need to target the midge larvae in the mud/detritus at the bottom of lakes/ponds, application approaches typically do not involve overhead sprays where exposure to the spray is a concern.

11) **Where can I get more information/studies regarding Bti and Bactimos WG?**

a. World Health Organization – Environmental Health Criteria 217 for *Bacillus thuringiensis* (http://www.who.int/ipcs/publications/ehc/en/EHC217.PDF?ua=1)
b. What is Bti? Q&A list on Bti from the Environmental Protection Agency (EPA) (https://www.epa.gov/mosquitocontrol/bti-mosquito-control).
c. World Health Organization specifications for Bti strain AM65-52 (http://www.who.int/whopes/quality/Bti_eval_spec_Jun_07.pdf)
d. Bactimos product brochure (https://publichealth.valentbiosciences.com/docs/public-health-resources/bactimos-sup-sup-pt---technical-use-bulletin_12-2-11)
e. World Health Organization's (WHO) Approved Mosquito Larvicides Products Table. The WHO has approved the use of Bti in drinking water (http://www.who.int/water_sanitation_health/gdwqrevision/Fourth_Edition_Bacillus_thuringiensis_Apr2010.doc)
f. Bactimos WG US Environmental Protection Agency Label (https://publichealth.valentbiosciences.com/docs/public-health-resources/bactimos-wg-specimen-label.pdf)

12) References cited in this FAQ and other midge control related publications

a. Ali A. 1995. Nuisance, economic impact and possibilities for control. In: The Chironomidae – the biology and ecology of non-biting midges (eds. Armitage P, Cranston PS, Pinder LCV). Published by Chapman & Hall, pp. 339-364.
b. Anonymous. 1977. Economic impact statement, blind mosquito (midge) task force. Sanford Chamber of Commerce, Seminole County, Florida.
c. Armitage, PS, Cranston PS & Pinder LCV (Eds) 1995. The Chironomidae. Chapman and Hall, London. 572 pgs.
d. Boisvert M, Boisvert J. 2000. Effects of Bti on target and nontarget organisms. Biocontrol Science and Technology. 10: 517-561.
e. Frouz J, Lobinske R, Yaqub A, Ali A. 2007. Larval gut profile in pestiferous *Chironomus crassicaudatus* & *Glyptotendipes paripes* in reference to the toxicity potential of Bti. JAMCA 23(3):355-358.
f. Lobinske R, Ali A, Frouz J. 2002. Ecological studies of spatial and temporal distributions of larval Chironomidae with emphasis on *Glyptotendipes paripes* in three central Florida lakes. Environ. Entomol.31:637-647.