



TECHNICAL USE BULLETIN

VectoBac®

Biological Larvicide

The science of specificity™



 For Organic Production

VectoBac® Biological Larvicide is a mosquito larvicide that contains the time-proven and environmentally compatible bacterial active ingredient *Bacillus thuringiensis* subsp. *israelensis* strain AM65-52. After 30 years of field use in a variety of settings around the globe, VectoBac has helped more people stay healthy and enjoy their quality of life in more communities worldwide than any other biorational mosquito larvicide.



Features and Benefits

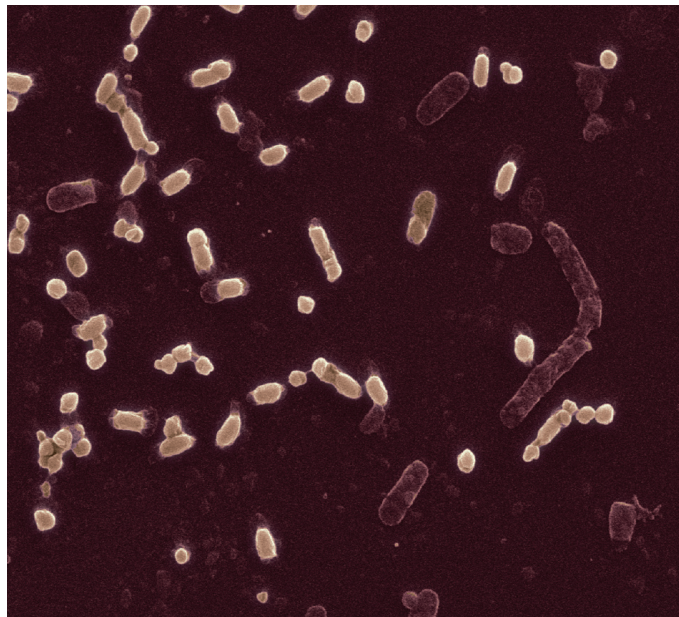
FEATURES	BENEFITS
Biorational larvicide	• Not harmful to non-target organism populations
Biological larvicide mode of action	
Broad control of all mosquito species	• Application flexibility
Available in various formulation types	
Quickly kills mosquito larvae (2–24 hours)	• Results observed quickly in the field
Organic Material Review Institute (OMRI) and National Organic Program (NOP) certified (select formulations)	• Peace of mind when treating sensitive mosquito larval habitats
Virtually dust-free dry formulations	• Less respirable and particulate dust

History

Bti is a naturally occurring spore-forming bacterium found in soil and aquatic environments throughout the world. At the time of sporulation, *Bti* produces a highly specific delta-endotoxin that is toxic upon ingestion only to the larvae of mosquitoes, black flies, and closely related flies. With over 30 years of field use in a variety of settings around the globe, *Bti* has been shown to provide effective, reliable and environmentally compatible control of mosquito larvae. In addition to the effectiveness of *Bti*, it has an excellent safety record and very low mammalian toxicity: LD50 values for both oral and dermal toxicity are more than 30,000 mg/kg. The mosquitocidal crystal proteins, spores and vegetative cells of *Bti* administered by different routes have been found to be non-pathogenic and non-toxic to various animal species in maximum challenge tests.¹ *Bti* is safe for use in aquatic environments, including drinking water reservoirs, for the control of mosquito, black fly and nuisance insect larvae.²

1. Becker N, Petric D, Zgomba M, Boase C, Dahl C, Lane J and A Kaiser. 2003. Mosquitoes and their control. Kluwer Academic; Plenum Publishers, New York: ISBN 0-306-47360-7.

2. Lacey LA and RW Merritt. 2003. The safety of bacterial microbial agents used for black fly and mosquito control in aquatic environments. In: "Environmental Impacts of Microbial Insecticides: Need and Methods for Risk Assessment" (HMT Hokkanen and AE Hajek, eds.), pp 151-168. Kluwer Academic Publishers Dordrecht, The Netherlands.



The Hidden Benefits of *Bacillus Thuringiensis* Subsp. *Israelensis*

For more than 30 years, *Bti* has played a pivotal role in public health programs by helping to control vector and nuisance insects around the world. Discovered and isolated in the 1970s, *Bti* is proven as an effective larvicide in the fight against mosquitoes and black flies (*Simulium*) while avoiding harm to non-target populations and the environment.

A fundamental challenge in public health—as in any situation where an active ingredient (AI) is used to control diverse populations of living organisms—is the concern about the onset of insect resistance. Resistance carries with it a

number of logistical and financial complications, including the need to:

- Increase application rates (accelerating the resistance process)
- Increase application frequency (accelerating resistance and increasing costs)
- Seek new solutions (which require additional investments of time and funding for long-term implementation)

What makes *Bti* significant in this regard?

Consider that in the more than 30 years since it was introduced to the public health domain, no commercially available *Bti* formulation has ever demonstrated operational-level resistance. Not a single case. How can this be?

Intrinsic Resistance Management: The Science Behind *Bti*

The reason *Bti* has remained effective since its introduction is the synergistic nature of four protein toxins that give *Bti* its efficacy. These four “protoxins” belong to three distinct toxin classes, each of which *Bti* releases when ingested by target larvae:

- Cyt1A (27 kDa)
- Cry4A (134 kDa)
- Cry4B (128 kDa)
- Cry11A (66 kDa)

While it's true that studies in a laboratory setting have shown resistance potential when individual toxins were isolated¹ from a particular strain of *Bti*,² no empirical evidence of resistance has ever been substantiated when using whole *Bti*—a fact that has been documented by many of the foremost public health scientists in the world.³ For this reason, it is not uncommon to hear *Bti* referred to as the single most important active ingredient available for public health larviciding programs.

To put it simply, *Bti* has resistance management qualities “built in.” Unlike other chemical or biochemical active ingredients used in public health pesticide products, *Bti* products provide not only superior efficacy, but also offer intrinsic resistance management benefits that have strong implications as part of a larger resistance management effort.

Mode of Action

Bti produces complex crystal proteins known as protoxins during sporulation. When these proteins are applied to larval habitats of mosquitoes, the mosquito larvae ingest them by filter feeding. The crystal proteins are solubilized by the alkaline juices in the larval midgut and are cleaved by the midgut proteases, yielding active peptide toxins called delta-endotoxins. The delta-endotoxins cause the formation of holes in the midgut cell wall, leading to lysis of cells and larvae death within 2–24 hours.

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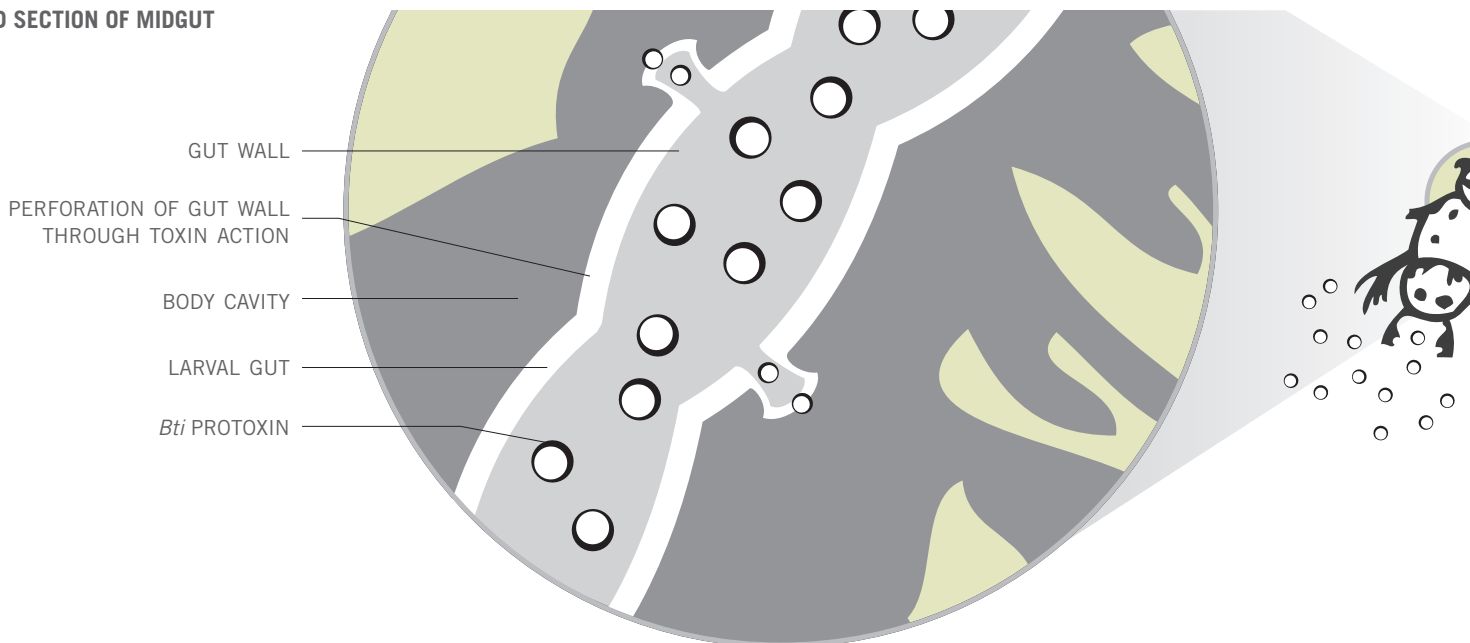
While studies in a laboratory setting have shown resistance potential when individual toxins were isolated³ from a particular strain of *Bti*,⁴ no empirical or operational evidence of resistance has ever been substantiated when using the naturally occurring wild-type *Bti* strain AM65-52—a fact that has been documented by many of the foremost public health scientists in the world.⁵ For this reason, it is not uncommon to hear *Bti* (including AM65-52) referred to as the single most important active ingredient available for public health larviciding programs.

3. Wirth. "Mosquito resistance to bacterial larvicidal toxins." *The Open Toxicology Journal*, 2010, 3:126–140.

4. Paul A, et al. "Insecticide resistance in *Culex pipiens* from New York." *Journal of the American Mosquito Control Association*, 2005, 21(3):305–309.

5. Becker N, Ludwig M. "Investigations on possible resistance in *Aedes vexans* field populations after a 10-year application of *Bacillus thuringiensis israelensis*." *Journal of the American Mosquito Control Association*, June 1993, 9(2):221–224.

ENLARGED SECTION OF MIDGUT



VECTOBAC MODE OF ACTION

- Mosquito larvae ingest *Bti* protoxin
- Protoxin activated in alkaline environment of the midgut
- Larval proteolytic enzymes break down activated protoxin into polypeptide fractions
- Polypeptide fractions act on midgut cells
- Midgut cells lyse
- Larvae die

Low Risk, Environmentally Compatible

ORGANISM	STUDY TYPE	RESULT
Odonata		
Dragonflies/Damselflies		
<i>T. corruptum</i>	Lab/naiads fed infected larvae	No effect
<i>E. civile</i>	Lab/naiads fed infected larvae	No effect
Ephemoptera		
Mayflies		
<i>C. pacificus</i>	Field treatment (<i>Bsph</i> technical powder 0.22 kg/ha)	No effect
Heteroptera		
Corixids/Notonectids		
<i>C. decolor</i>	Field treatment (<i>Bti</i> technical powder 0.56 kg/ha)	No effect
<i>N. undulata</i>	Lab/fed infected larvae	No effect
<i>N. unifasciata</i>	Field study/treated ponds	No effect
<i>Buenoa spp.</i>	Field study/treated ponds	No effect
Coleoptera		
Dytiscidae	Field studies	No effect
Hydrophilidae	Field studies	No effect
Crustacea		
<i>Daphnia spp.</i>		
<i>E. bampo</i>	Laboratory	100-200X mosquito rate
Crawfish		
<i>P. clarkii</i>	Laboratory	Effect at 1,000X mosquito rate

Lacey and Mulla (1990). Safety of *Bacillus thuringiensis* subsp. *israelensis* and *Bacillus sphaericus* to non-target organisms in the aquatic environment. In "Safety of Microbial Insecticides" (Marshall Laird, Lawrence Lacey, and Elizabeth Davidson eds.), Chap. 12. CRC Press, Inc. Boca Raton, Florida.

Bti has been extensively tested and is not a human health hazard when handled as instructed by the product label.

Building on the Benefits of Intrinsic Resistance Management

Biologists know there is no such thing as a silver bullet. While *Bti* has built-in resistance management properties that make it an indispensable tool for sustainable mosquito control programs, *Bti* products as “stand-alone” solutions do have some limitations:

- The same qualities that make *Bti* a low-impact product for the environment also limit its residual activity in natural water bodies. *Bti* breaks down quickly, meaning that most *Bti* applications made to open water bodies provide control of mosquito larvae for a limited amount of time.
- *Bti* is proven to be extremely effective in “clean” water habitats, but higher rates are needed in habitats with more organic content.
- *Bti* is effective only against mosquito larvae (1st through mid-4th instar). As such, the use of *Bti* requires dedicated site surveillance to ensure that applications are made within the appropriate treatment window.

These limitations can be overcome, however, by formulating *Bti* in combination with other AIs that have greater residual efficacy, such as *Bacillus sphaericus* (*Bsph*). Although *Bsph* carries the potential for resistance development, the intrinsic resistance management properties of *Bti* can prevent that resistance from developing. The result is an elegant, synergistic solution for longer lasting, broader spectrum mosquito control while addressing resistance concerns.⁴

In fact, combinations with *Bti* have been shown to restore or “bring back” susceptibility to *Bsph* in cases where resistance has developed.⁵ This synergistic effect occurred only when the AIs were combined, as opposed to being used separately in rotation.⁴

Bti: The Bottom Line

With intrinsic resistance management provided by *Bti*, mosquito abatement programs are able to standardize their operations with fewer products while at the same time maintaining best practices for resistance management. That means simplified operations: fewer decisions, fewer calibrations/characterizations for application equipment, and increased opportunities to maximize efficiency, cost-effectiveness, and expertise.

It's a welcome combination that has made *Bti* the number-one biological mosquito larvicide around the globe. And for an industry that has traditionally had a limited number of tools, *Bti*'s intrinsic resistance management qualities show tremendous promise as a sustainable solution not only today, but for the long term.

1. Wirth. “Mosquito resistance to bacterial larvicidal toxins.” *The Open Toxicology Journal*, 2010, 3:126–140.

2. Paul A, et al. “Insecticide resistance in *Culex pipiens* from New York.” *Journal of the American Mosquito Control Association*, 2005, 21(3):305–309.

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4. Zahiri NS, Mulla MS. “Susceptibility profile of *Culex quinquefasciatus* (Diptera: Culicidae) to *Bacillus sphaericus* on selection with rotation and mixture of *B. sphaericus* and *B. thuringiensis israelensis*.” 2003, *J Med Entomol* 40:672–677.

5. Zahiri NS, Su TY, Mulla MS. “Strategies for the management of resistance in mosquitoes to the microbial control agent *Bacillus sphaericus*.” 2002, *J Med Entomol* 39:513–520.

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Protecting Life's Potential

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Valent Health

1910 Innovation Way, Suite 100
Libertyville, Illinois 60048

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