

The science of specificity™



VectoBac® Bacterial 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

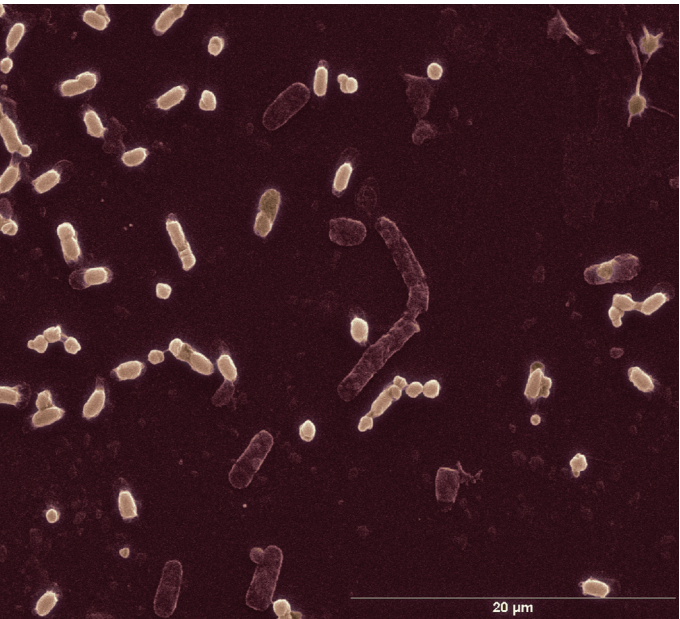
FEATURES	BENEFITS
Biorational larvicide	• Not harmful to non-target organism populations
Bacterial larvicide mode of action	
Broad control of all mosquito species	• Application flexibility
Available in various formulation types	
Quickly kills mosquito larvae (2–24 hours)	• Results observed quickly in 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.



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.

The reason Bti has remained effective since its introduction is the synergistic nature of four protein toxins that give Bti its efficacy. These four delta-endotoxins 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)

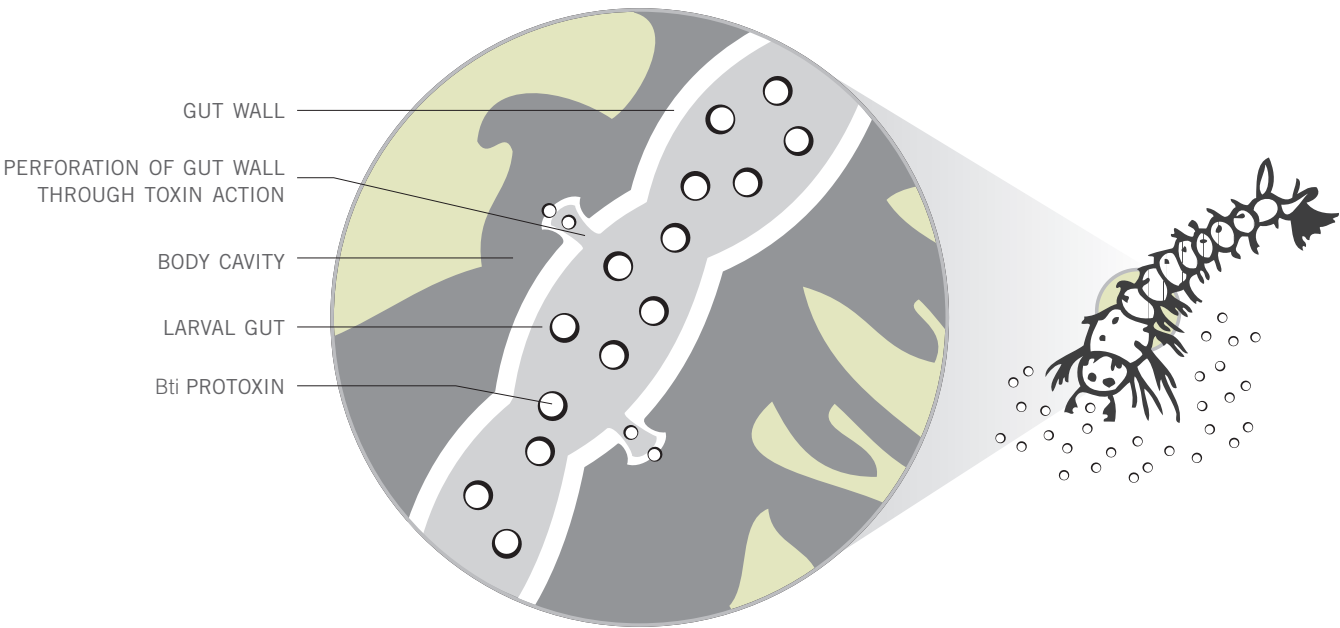
While studies in a laboratory setting have shown resistance potential when individual toxins were isolated³ from a particular strain of Bti,⁴ no empirical or operational evidence of resistance has ever been substantiated when using the naturally occurring wild-type Bti strain AM65-52—a fact that has 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 Toxinology 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 (Bti 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.

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.

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 Toxinology 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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