VALENT HEALTH™
Protecting Life's Potential

TECHNICAL USE BULLETIN

VectoLex®

Bacterial Larvicide

The power of residual efficacy™

VectoLex® BACTERIAL LARVICIDE

VectoLex® Bacterial Larvicide is a mosquito larvicide that contains the time-proven and environmentally compatible bacterial active ingredient *Bacillus sphaericus* (strain ABTS-1743)V. ectolex helps control mosquito populations in both clean and polluted waters with the industry's most target-specific biorational for residual control of West Nile virus vectors.

Features and Benefits

FEATURES	BENEFITS
Biorational larvicide Industry's most target-specific mosquito larvicide	• Not harmful to non-target organism populations
Bacterial larvicide mode of action	• Results can be assessed in the field
Vectolex provides up to 28 days of residual control*	• Reduced number of applications
Can be used in clean and polluted habitats **Available in multiple formulation types** **Vectolex WSP easy to apply into catch basins** (malleable, slips into tight spots)	• Application flexibility
National Organic Program (NOP) listed (for select formulations)	• Peace of mind when treating mosquito larval habitats on organic farms
Vectolex WSP initially releases product/activity from water surface	• Does not get bound up in debris or sludge at the bottom of catch basins
Virtually dust-free granule and water dispersible granule formulations	• Less respirable and particulate dust
Dust-free catch basin option	• Eliminates cleanup of PPE
Uniform carrier for granule formulation	• Even applications with no bridging at lower application rates

*Length of control dependent on local conditions and rate used.

History

BACILLUS SPHAERICUS,
STRAIN ABTS-1743

The following table derived from de Barjac and Sutherland's book, entitled *Bacterial Control of Mosquitoes and Black Flies. Biochemistry, Genetics and Applications of* Bacillus thuringiensis israelensis *and* Bacillus sphaericus, highlights the history of the development of key *Bsph* strains.

KEYS TRAINS	HISTORICAL SIGNIFICANCE OFF KEY STRAINS	OTHER STRAINS AND RELATIONSHIP TO KEY STRAINS
K (U.S.)	First reported active isolate; work discontinued after brief program	Q (U.S.); Phage group I; low larvicidal activity
SSII-1 (India)	First active isolate universally available; fermentation and population stability problems	1404 (Philippines); 1883, 1885-1893, 1895, 1896 (Israel); Phage group 2; moderate larvicidal activity
1593 (Indonesia)	First fermentation- and populationstable strains; one of key field candidates	1691, 1881 (El Salvador); 2013-4, 2013-6 (Romania); 2117-1 (Philippines); 2500, 2501 (Thailand); Phage group 3; high larvicidal activity
2362 (Nigeria)	First highly active African strain; the prime field candidates	
Lysenko Isolates: 2537-2, 2533-1 (KI) 2533-1 (K2) (Guyana) 2601 (Hungary) 2602 (Czechoslovakia)	First active types isolated from non-mosquito terrestrial sources	
2297 (Sri Lanka)	Crystal first noted in this strain of key field candidates	2173, 2377 (India), 2317-3 (Thailand); field candidates Phage group 4; mixed larvicidal activity; 1894 (Israel); Phage group 5; 2115 (Philippines); Phage group 6; 2315 (Thailand); Phage group 7; moderate larvicidal activity

Strain Specificity

HISTORY: *BACILLUS SPHAERICUS* STRAIN ABTS-1743 (CONTINUED)

By 1988, 186 strains had been isolated worldwide, of which 45 had shown some toxicity to mosquito larvae (the most commercially interesting strains were 1593, 2297 and 2362). A number of these isolates were maintained at the World Health Organization (WHO) Collaborating Centre at the Institute Pasteur, Paris, and were available to scientists for many years,

The Bsph 2362 strain was received as a slant culture by Abbott Laboratories (former parent company of Valent BioSciences Corporation-VBC) in 1982, and the first lyophilized batch was a mass transfer of sporulated cells. Over the course of the 1980s, this strain was preserved for long-term use and is currently used to provide inoculum for large-scale commercial fermentation of Bsph by VBC. In 1986, the 2362 strain was provided a unique strain number, ABTS-1743, by Abbott Laboratories.

In 1991, Abbott received registration of Bsph by the US Environmental Protection Agency (U.S. EPA). The registration package submitted to the U.S. EPA designated the WHO strain 2362 as strain ABTS-1743. However, the U.S. EPA registered the technical product as strain 2362 at that time. As such, for over 20 years Bsph strain 2362 has been associated with VB C's VectoLex (Bacillus sphaericus 2362, strain ABTS-17 43) and VectoMax® (Bacillus thuringiensis subsp. israelensis, strain AM65-52 + Bacillus sphaericus 2362, strain ABTS-1743) brands worldwide and is recognized in the literature as associated with VBC. In 2010, the U.S. EPA decided that the 2362 designation is a ubiquitous strain identifier and belongs to the WHO; as such, no single company can claim exclusive use of this strain in the U.S. Thus VBC was mandated to amend its registration by adding the strain isolate number ABTS-1743 to the active ingredient name; the same strain isolate number that was submitted to the U.S. EPA as part of the original registration dossier for Bsph in the 1980s. In addition, the U.S. EPA mandated that all registrants of Bsph products amend their registrations to provide a unique strain.

Factors Affecting Performance

Vector control professionals will find that the performance of VectoLex is affected by factors similar to those of other bacterial larvicides.

LARVAL STAGES

Larvae in the early stages of development are more susceptible to a given quantity of VectoLex than are those in later stages. When VectoLex is ingested by 1st, 2nd and 3rd instar larvae, it is very efficacious, even under conditions of rapid larval development. If late 3rd and early 4th in star larvae predominate, higher rates shou Id be used.

WATER QUALITY

The higher recommended rates of VectoLex should be used in water with high organic content. This will compensate for the larave's ingestion of a smaller proportion of active ingredient relative to the overall food supply.
Other water quality parameters, such as salinity or pH, have little effect on the activity of VectoLex. Water temperature, because it influences larval metabolism, can be a factor in determining application rates. Lower temperatures may reduce feeding activity, and thus efficacy, at lower application rates.

RESIDUAL ACTIVITY

VectoLex offers up to 28 days of residual control in many habitats. Major drivers of this residual efficacy are due to 1) Bsph protoxins and spores remaining suspended in the water column for extended periods of time, and 2) recycling of the bacteria in dead larvae. Duration of control will depend upon habitat factors such as flushing, water chemistry and frequency of oviposition to maintain the recycling process. Populations should be monitored and applications made as necessary.

Mode of Action

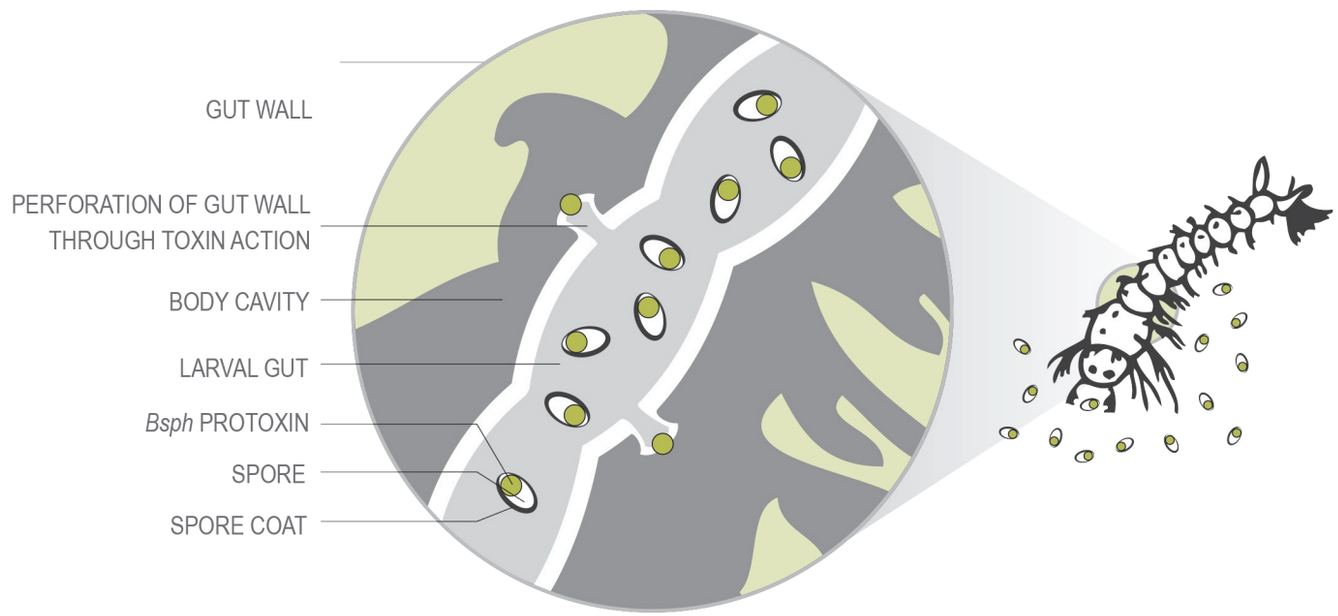
Bsph produces complex crystal proteins known as protoxins during sporulation. When these proteins are applied to larval habitats of mosquitoes, the mosquito larvae ingest them by filter feeding. The crystal proteins are solubilized by the alkaline juices in the larval midgut and are cleaved by the midgut 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.

Unlike Bacillus thuringiensis subsp. israelensis (Bti), the Bsph toxin and bacteria I spore a re enclosed within a spore coat. In addition, the Bsph toxins bind to chemically different receptor sites on the midgut cells relative to Bti. In fact, Bti and Bsph are not related immunologically and are thought to have completely different molecular modes of action.

VectoLex should be applied during the 1st through early 4th larval instar. During the later part of the 4th instar, the larvae are no longer eating. Compared with Bti, larval mortality after ingesting Bsph will take slightly longer to achieve. However, even larvae that survive an initial exposure to the Bsph toxin exhibit long-lasting effects that limit the potential for a normal life span. 1 Fewer survivors can successfully pupate, and survivors that develop to the adult stage have lower nutritional reservesanother advantage of VectoLex.

1. Zahiri NS, Mu Ila MS. 2004. Non-larvicidael ffectso f Bacillust huringiensis israelensisa nd Bacillus sphaericus on oviposition and adult mortality of Culex quinquefasciatus Say (Diptera, Culicidae). Journal of Vector Ecology30(1): 155-162.

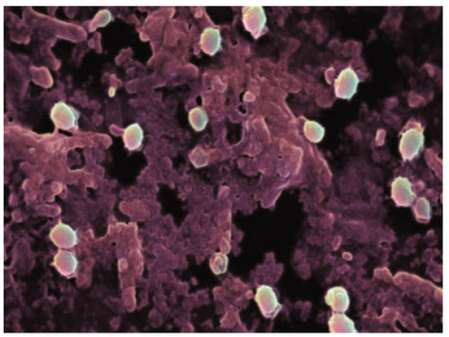
ENLARGED SECTION OF MIDGUT



VECTOLEX MODE OF ACTION

- Mosquito larvae ingest *Bsph* 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

Bacillus Sphaericus Advantages



Bacillus sphaericus (strain ABTS-1743) is a natu ra l ly occurring spore-forming bacterium found throughout the world in soil and aquatic environments. Extended residual activity in the environment is one of the outstanding properties of Bsph. The delta-endotoxin of Bsph persists in the water column and is available for larvae to feed on longer relative to other bacterial larvicides; even in highly organic aquatic environments. This persistence is thought to be the result of a combination of features, including protection of the protein by the spore coat; slower settling rate; and the unique ability of Bsph spores to germinate, grow and produce toxins in cadavers of mosquito larvae treated with the materia l. Often, the greater the larval population upon initial treatments, the greater the residua l effect of Bsph.

The other outstanding property of Bsph is its specificity toward mosquitoes. In fact, Bsph is the only mosquito larvicide to be developed specifically for the mosquito control industry (i.e., Bsph is not a derivative from the agricultural sector). Bsph is not effective against other insect orders and is not operationally effective against other Dipteran larvae. This makes Bsph unique and provides the industry with the most targetspecific method for controlling mosquitoes today.

Strain Specificity

WHY STRAIN NUMBERS ARE IMPORTANT

Bacterial larvicides such as Bti and Bsph are like all living organisms: no two "cells" are perfectly identical. While genetics plays a crucial role, the environment in which a bacterium grows can have a significant impact on protoxin expression and therefore performance. This is analogous to identical twins being separated at birth. 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. The manufacturer's unique strain number for bacterial-based biopesticides is a critical link to product performance and quality expectations. The published literature shows that identical strains produced under different fermentor conditions can affect performance. Even when the same strain is used, differences or changes in the use of raw materials, fermentation, recovery and formulation processes wi 11 greatly affect not only the quality of end-use product, but will also likely affect the biological performance. 1 Fermentation recovery methods can be different from manufacturer to manufacturer (e.g., precipitation, centrifugation, ultrafiltration) and can ultimately affect critical factors such as particle size, which drives behavior in the water column and the increased probability of the insect ingesting the toxin. 1•2•3 Furthermore, since this is a fermentation process, sub-standard quality controls can lead to undesirable growth of contaminants that could cause end-user and environmental safety hazards. In addition, quality control (QC) requirements for fermentation are critical to ensure consistency and safety. The same QC measures are not utilized by all manufacturers of bacterial-based larvicides and as such, it is critical to differentiate these products based on the manufacturer's unique strain number from ubiquitous strain identifiers (e.g., using H 14 as an "identifier" for Bti-based products). Use of ubiquitous strain identifiers does not guarantee identical product performance or environmental safety.

1. Devisetty BN. 1993. Production and formulation aspects of Bacillus thuringiensisl.n : Proceedingso f the 2nd CanberraM eetingo n Bacillus thuring1ensis(Akhurst RJ, editor). Australia, CPN Publications Pty. Ltd.

2. Clark JD, Devisetty BN, Krause SC, Novak RJ, Warrior P. 2006. Particled istributionabl ehavioro f a spray-driedt echnicalc oncentrate and a water-dispersibleg ranulef ormulationo f Bacilluss phaericusi n an aqueous column. J Am Mosq Control Assoc. (4PI8-724.

3. Clark JD, Devisetty BN, Krause SC, Novak RJ, Warrior P. 2007. A novel methodf or evaluatingt he particled istributionabl ehavioro f a spray-dried technicalc oncentratea nd a water-dispersiblge ranulef ormulationo f Bacillust huringiensiss ubspi srae!ensisin an aqueousc olumn.J Am Mosq Control Assoc. 23(n60-65.

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Low Risk, Environmentally Compatable

ORGANISM	STUDY TYPE	RESULT
Odonata		
Dragonflies/Damselflies		
T. corruptum	Lab/naiads fed infected larvae	No effect
E. civile	Lab/naiads fed infected larvae	No effect
Ephemoptera		
Mayflies		
C. pacificus	Field treatment (*Bsph* technical powder 0.22 kg/ha)	No effect
Heteroptera		
Corixids/Notonectids		
C. deco/or	Field treatment (*Bsph* technical powder 0.25 kg/ha)	No effect
N. undulata	Lab/fed infected larvae	No effect
A. bouvieri	Lab/LC50	500X mosquito LC50
N. unifasciata	Field study/treated ponds	No effect
Buenoa spp.	Field study/treated ponds	No effect
Coleoptera		
Dytiscidae	Field studies	No effect
Hydrophilidae	Field studies	No effect
Crustacea		
Daphnia		
D. similis	Laboratory	Effect at 27, 000X mosquito rate
Fairy Shrimp	Laboratory	
S. dichotomus		Effect at 15,000X mosquito rate
Crawfish		
P clarkii	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.

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

To learn more about **VectoLex**, scan the following **QR code**:

VALENT HEALTH™
Protecting Life's Potential

Valent Health is an ISO 9001 Certified Compay

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