



TECHNICAL USE BULLETIN

VectoLex[®]

Bacterial Larvicide

The power of residual efficacy™



VectoLex® Bacterial Larvicide is a mosquito larvicide that contains the time-proven and environmentally compatible bacterial active ingredient *Bacillus sphaericus* (strain ABTS-1743). VectoLex 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 | • Application flexibility |
| Available in multiple formulation types | |
| VectoLex WSP easy to apply into catch basins (malleable, slips into tight spots) | |
| 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 *Bsp* strains.

| KEY STRAINS | HISTORICAL SIGNIFICANCE OF 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 1; 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 population-stable 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 (K1) 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); 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 *Bsp* 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 *Bsp* by VBC. In 1986, the 2362 strain was provided a unique strain number, ABTS-1743, by Abbott Laboratories.

In 1991, Abbott received registration of *Bsp* 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 *Bsp* strain 2362 has been associated with VBC's VectoLex (*Bacillus sphaericus* 2362, strain ABTS-1743) 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 *Bsp* in the 1980s. In addition, the U.S. EPA mandated that all registrants of *Bsp* 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 instar larvae predominate, higher rates should be used.

WATER QUALITY

The higher recommended rates of VectoLex should be used in water with high organic content. This will compensate for the larvae'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) *Bsp* 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

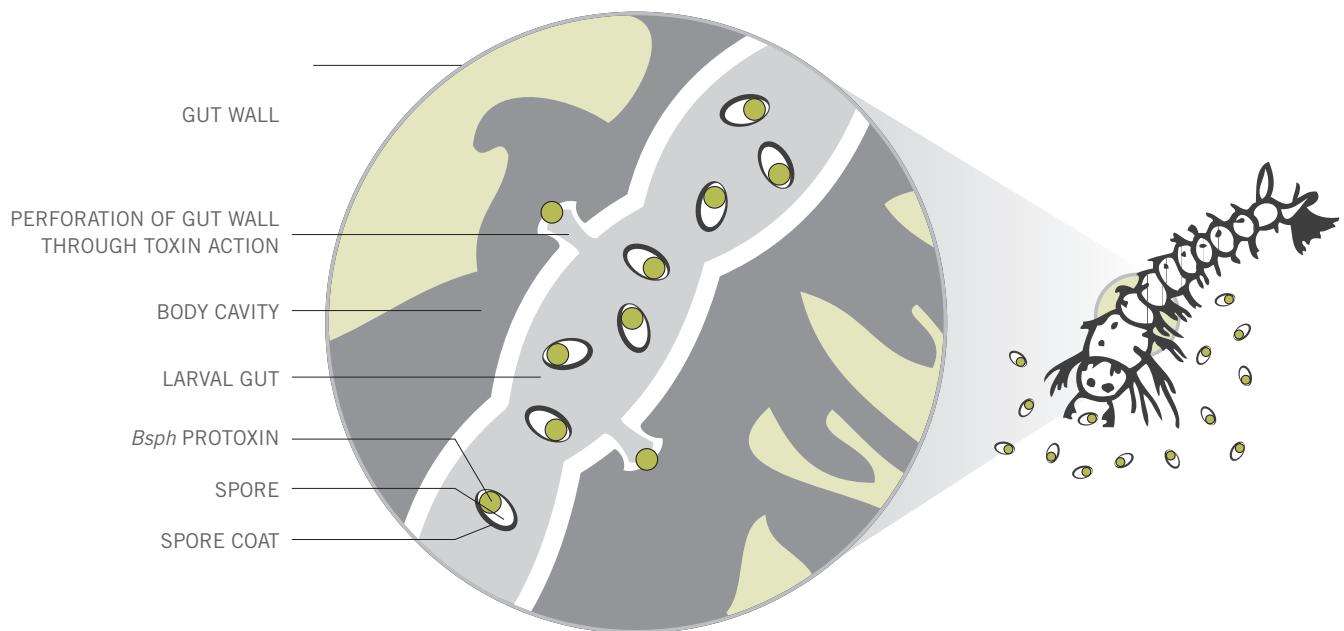
Bspf produces complex crystal proteins known as protoxins during sporulation. When these proteins are applied to larval habitats of mosquitoes, the mosquito larvae ingest them by filter feeding. The crystal proteins are solubilized by the alkaline juices in the larval midgut and are cleaved by the midgut 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 *Bspf* toxin and bacterial spore are enclosed within a spore coat. In addition, the *Bspf* toxins bind to chemically different receptor sites on the midgut cells relative to *Bti*. In fact, *Bti* and *Bspf* 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 *Bspf* will take slightly longer to achieve. However, even larvae that survive an initial exposure to the *Bspf* 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 reserves—another advantage of VectoLex.

1. Zahiri NS, Mulla MS. 2004. Non-larvicidal effects of *Bacillus thuringiensis israelensis* and *Bacillus sphaericus* on oviposition and adult mortality of *Culex quinquefasciatus* Say (Diptera: Culicidae). *Journal of Vector Ecology* 30(1): 155-162.

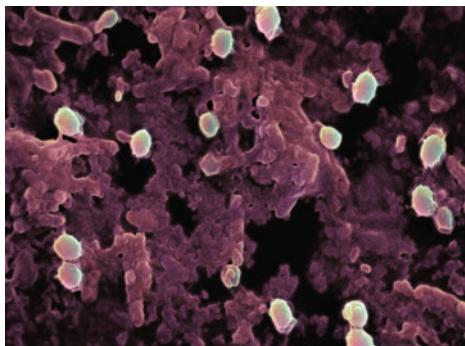
ENLARGED SECTION OF MIDGUT



VECTOLEX MODE OF ACTION

- Mosquito larvae ingest *Bspf* 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 naturally 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 *Bspf*. The delta-endotoxin of *Bspf* 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 *Bspf* spores to germinate, grow and produce toxins in cadavers of mosquito

larvae treated with the material. Often, the greater the larval population upon initial treatments, the greater the residual effect of *Bspf*.

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

Strain Specificity

WHY STRAIN NUMBERS ARE IMPORTANT

Bacterial larvicides such as *Bti* and *Bspf* 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 will 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 H14 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 thuringiensis*. In: Proceedings of the 2nd Canberra Meeting on *Bacillus thuringiensis* (Akhurst RJ, editor). Australia: CPN Publications Pty. Ltd.

2. Clark JD, Devisetty BN, Krause SC, Novak RJ, Warrior P. 2006. Particle distributional behavior of a spray-dried technical concentrate and a water-dispersible granule formulation of *Bacillus sphaericus* in an aqueous column. *J Am Mosq Control Assoc.* (4):718–724.

3. Clark JD, Devisetty BN, Krause SC, Novak RJ, Warrior P. 2007. A novel method for evaluating the particle distributional behavior of a spray-dried technical concentrate and a water-dispersible granule formulation of *Bacillus thuringiensis* subsp *israelensis* in an aqueous column. *J Am Mosq Control Assoc.* 23(1):60–65.

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>Bspf</i> technical powder 0.22 kg/ha) | No effect |
| Heteroptera | | |
| Corixids/Notonectids | | |
| <i>C. decolor</i> | Field treatment (<i>Bspf</i> technical powder 0.25 kg/ha) | No effect |
| <i>N. undulata</i> | Lab/fed infected larvae | No effect |
| <i>A. bouvieri</i> | Lab/LC50 | 500X mosquito LC50 |
| <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</i> | | |
| <i>D. similis</i> | Laboratory | Effect at 27,000X mosquito rate |
| Fairy Shrimp | | |
| <i>S. dichotomus</i> | Laboratory | Effect at 15,000X 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.

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

To learn more about **VectoLex**,
call **800.323.9597** or
scan the following **QR code**:



**Valent BioSciences Corporation is
an ISO 9001:2008 Certified Company**

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