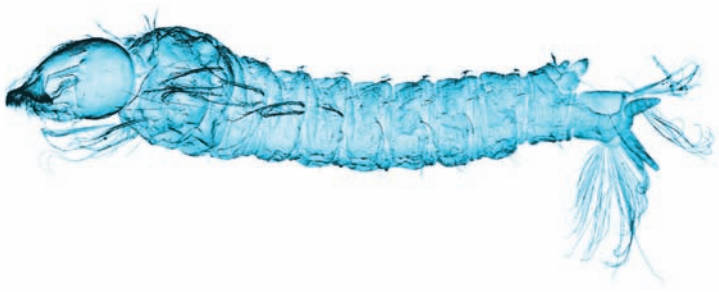
TECHNICAL USE BULLETIN

VectoMax™

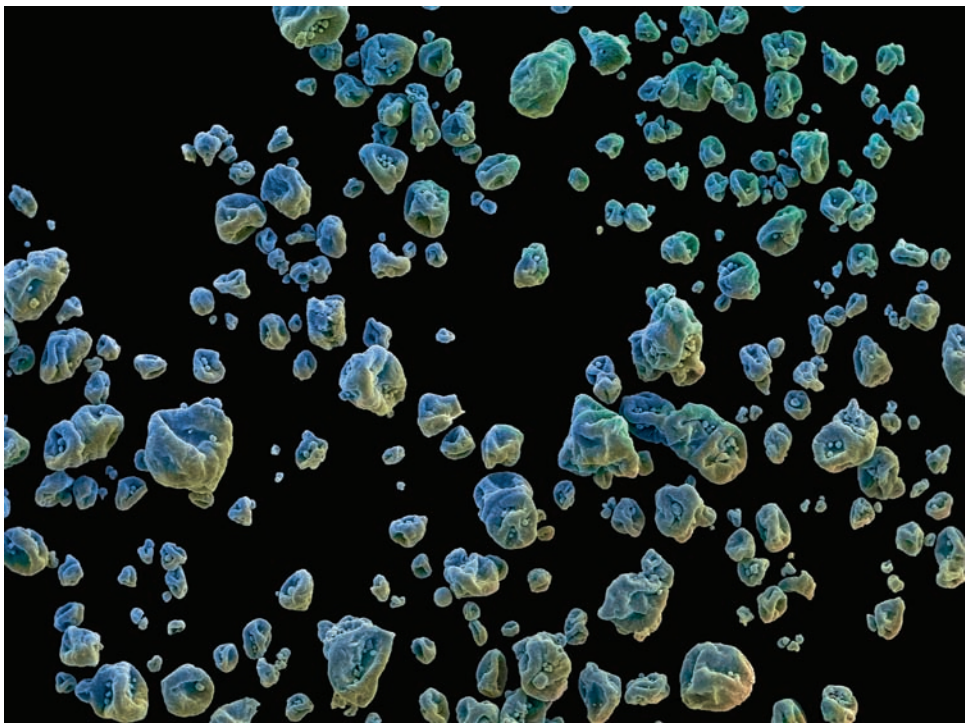
Biological Larvicide

BioFuse™ TECHNOLOGY

GRANULES



VALENT BioSciences Corporation



VECTOMAX™ TECHNICAL POWDER // Each particle contains microbial larvicide protoxins from *Bti* and *Bacillus sphaericus*.

VectoMax™ CG and VectoMax™ G Biological Larvicides are advanced biological mosquito larvicides based on Valent BioSciences Corporation's new proprietary BioFuse™ Technology, which combines the time-proven and environmentally compatible microbial active ingredients *Bacillus thuringiensis* subsp. *israelensis (Bti)* and *Bacillus sphaericus (Bsph)* **into a single micro particle.** Thus, mosquito larvae get a dose of a carefully selected ratio of both toxins when VectoMax™ particles are ingested. Consisting of only *Bti, Bsph* and food-grade (USEPA list 4) inert ingredients, VectoMax™ CG and G have received approval for application in organic crops and sensitive habitats.

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FEATURES

- Quickly kills mosquito larvae
- Controls all mosquito species
- Highly specific activity on mosquitoes
- Can be used in clean and polluted habitats
- Offers residual control of several mosquito species
- Up to 4 weeks of residual control under typical environmental conditions
- Combination microbial
- Uniform carrier
- Dry formulation

BENEFITS

- Results assessed quickly in the field
- Not harmful to non-target organisms
- Virtually dust free granules
- Even applications with no bridging at lower application rates

History

BACILLUS THURINGIENSIS SUBSP. *ISRAELENSIS* (STRAIN AM65-52)

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 only to larvae of mosquitoes, black flies, and closely related flies upon ingestion.

During 25 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 for the control of mosquito, black fly, and nuisance insect larvae.[2]

BACILLUS SPHAERICUS (STRAIN 2362)

Bsph is also a naturally occurring spore-forming bacterium found throughout the world in soil and aquatic environments. Early development of *Bsph* for mosquito control focused on strains isolated and maintained by the Pasteur Institute, WHO collaborating Center, Paris, France. Since 1995, *Bsph* has demonstrated the ability to provide residual control of mosquito larvae in a great variety of aquatic habitats. This biological larvicide is capable of providing residual control in highly organic environments, including catch basins, sewage effluent, sewage lagoons, oxidation ponds, animal waste lagoons, septic ditches, etc.

BIOFUSE™ TECHNOLOGY

Microbial mosquito larvicides have gained considerable acceptance in recent years. The value of *Bti* and *Bsph* to mosquito control programs worldwide is well established.[1] *Bti* and *Bsph* each offer unique advantages relative to chemical insecticides. Both *Bti* and *Bsph* offer relative safety to humans and non-target organisms.[2,3,4] *Bti* provides broad spectrum activity, rapid control, and low potential for resistance; while *Bsph* exhibits extended residual control, efficacy in polluted water, and high target specificity.[5]

Due to these unique advantages, Valent BioSciences Corporation developed BioFuse™ Technology, a proprietary (patent pending USA; patent approved EU) technology that combines *Bti* and *Bsph* in a specific toxin ratio into **every micro particle**. This technology offers mosquito control professionals the ability to take advantage of each microbial larvicide's strengths, while significantly reducing the limitations that each possesses.

[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.

[3] World Health Organization, 1999, Environmental health criteria 217 microbial pest control agent *Bacillus thuringiensis,* WHO, Geneva Switzerland, ISBN 92 4 157217 5.

[4] Siegel JP and DA Shadduk. 1990. Mammalian safety of Bacillus sphaericus, Chp. 21 in "Bacterial Control of Mosquitoes and Black Flies" (de Barjac & Sutherland eds.). Rutgers University Press, ISBN 0-8135-1546-7

[5] Lacey LA. 1990. Chp. 18 *In:* "Bacterial control of mosquitoes and black flies" (deBarjac H and DJ Sutherland eds.). Rutgers University Press, New Brunswick, NJ.

Mode of Action

Both *Bti* and *Bsph* produce 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.

However, activity of the delta-endotoxin for *Bsph* differs from that of *Bti* in several important ways. For *Bsph,* the toxin is attached to the bacterial spore, while *Bti* toxins are not attached to the spore (parasporal). The toxins of *Bsph* and *Bti* bind to chemically different receptor sites on cells. They are not related immunologically and are thought to have completely different molecular modes of action.

Operationally, the most important differences between the toxins of *Bsph* and *Bti* are speed of action and persistence in natural larval habitats. *Bsph* toxin is much slower-acting than *Bti* toxin. Larval mortality can take several days but is usually expressed within 48 hours of ingestion, while *Bti* provides quick kill. Initial results can be seen within 2–24 hours. *Bsph* toxin is also much more persistent in natural larval habitats than *Bti.* 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 material. VectoMax™ CG and G combine the quick kill seen with *Bti* **in combination** with the residual properties of *Bsph.*

Application

VectoMax™ CG is available in 10/14 mesh, and VectoMax™ G is available in 5/8 mesh. The granules are designed to be applied by ground or aerial application.

The size, shape, and density of the granules lessen the potential for off-target application due to aerial drift and enable good penetration of dense vegetation. The presence of both floating and sinking granules helps ensure the distribution of the active ingredient throughout the larval feeding zone.

BULK DENSITY

Bulk density should be measured with multiple lots to insure accurate data. (This varies somewhat with relative humidity and each batch.) However, the following can be used as a general guideline:

VectoMax™ CG 10/14 mesh	VectoMax™ G 5/8 mesh
Approximately 30 lbs/ft^3 or 480 kg/m^3	Approximately 25 lbs/ft^3 or 401 kg/m^3

VectoMax™ granules are available in the standard pack size of 40 lb (18.1 kg) bags. Special bulk orders are available in 1,200 lb super sacks (544 kg).

GROUND APPLICATIONS

VectoMax™ Biological Larvicide granular treatments can be made with many types of ground equipment designed for granule application. These include manually or mechanically driven devices relying on a whirling disk (e.g. Cyclone® seeder, Ortho Whirlybird® seeder), and air-blast applicators (Buffalo® turbine, Maruyama® or Stihl® power backpacks). Vectomax™ CG is suitable for Horn seeder applications.

When using VectoMax™ granules, it is important to properly calibrate application equipment. Granule output at a given setting should be determined, as well as swath width and required speed of travel.

Consult your Valent BioSciences® Technical Specialist to determine optimal application methods to meet your program objectives.

AERIAL APPLICATIONS

VectoMax™ granules can be applied aerially with conventional fixed-wing aircraft or helicopter granule application equipment. Each application unit should be calibrated and the swath characterized using VectoMax™ granules before being used operationally. The variety of equipment in field use precludes specific instructions on settings, airspeed, etc. As an example: ram-air type of applications used on most fixed-wing aircraft usually requires a simple adjustment of the baffle plate or gate to decrease the granule flow rate. Most aerial treatment rates will be in the range of 5-10 pounds of VectoMax™ granules per acre (5 to 10 kg of VectoMax™ granules per hectare).

APPLICATION RATES

I. For control of mosquito larvae species* in the following non-crop sites:

HABITAT	RATE/RANGE
Wastewater: Sewage effluent, sewage lagoons, oxidation ponds, septic ditches, animal waste lagoons, impounded wastewater associated with fruit and vegetable processing.	5-20 lbs/acre*
Storm Water/Drainage Systems: Drainage ditches, roadside ditches, retention, detention, and seepage ponds.	5-20 lbs/acre*
Marine/Coastal Areas: Tidal water, salt marshes, mangroves, estuaries.	5-20 lbs/acre*
Water Bodies: Natural and manmade aquatic sites such as lakes, ponds, canals, rivers and streams (including river and stream edges), floodplains, swamps, marshes, irrigation ditches, floodwater, woodland pools, snow melt pools, and livestock watering ponds and troughs.	5-20 lbs/acre*
Waste Tires: Tires stockpiled in dumps, landfills, recycling plants, and similar sites.	20-80 lbs/acre[1]

[1]0.5-2 lbs/1,000 sq. ft.

II. For control of mosquito larvae in agricultural/crop sites where mosquito breeding occurs.

HABITAT	RATE/RANGE
Rice fields, pastures/hay fields, orchards (including citrus groves, peaches, almonds, dates, walnuts), asparagus fields, corn fields, cotton fields, alfalfa fields, vineyards.	5-20 lbs/acre*

Apply uniformly by aerial or conventional ground equipment. Reapply as needed (up to 4 weeks under typical environmental conditions).

* Use higher rates (10 to 20 lbs/acre) in areas where 4th instar *Aedes* or *Ochierotatus* spp. larvae predominate, or very high densities of late instar larvae are present, or under conditions where local experience indicates the need for higher rates to achieve extended residual control.

The following table can be used as a guide in determining rates of VectoMax™ granule application:

RATE IN POUNDS/ACRE (KILOGRAMS/HECTARE)	VECTOMAX™ G 5/8 MESH		VECTOMAX™ CG 10/14 MESH	
	Ft^2	M^2	Ft^2	M^2
2.5 (2.8)	2	18	10	104
5.0 (5.6)	3	36	19	207
7.5 (8.4)	5	55	29	311
10.0 (11.2)	7	73	39	414
	65 granules = 1 gram		370 granules = 1 gram	

Note: granules per gram and number of granules per ft^2/m^2 are approximations.

Storage

When stored in a cool, dry place, out of direct sunlight, useful life is expected to be greater than two seasons.

Precautions should be taken to provide a long-term storage area that is dry, with average temperature below 24˚C (75˚F) and rodent-proof. In northern areas, product can be held in unheated storage facilities. Freezing will not reduce potency. In warm climates, ambient storage (> 24˚C) for 1-2 years will not reduce efficacy.

Container Disposal

Non refillable container. Do not reuse or refill this container. Completely empty bag into application equipment by shaking and tapping sides and bottom to loosen clinging particles. Then offer for recycling, if available, or dispose of empty bag in a sanitary landfill or by incineration. Do not burn unless allowed by state and local ordinances.

Do not contaminate water, food, or feed by storage or disposal.

Effects on Non-Target and Beneficial Organisms

Both *Bti* and *Bsph* have excellent safety records and very low mammalian toxicity. Effects of label-approved applications on non-target organisms are practically nonexistent.[1]

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 (*Bti* technical powder 0.56 kg/ha)	No effect
C. pacificus	Field treatment (*Bsph* technical powder 0.22 kg/ha)	No effect
Heteroptera		
Corixids/Notonectids		
C. decolor	Field treatment (*Bti* technical powder 0.56 kg/ha; *Bsph* technical powder 0.25 kg/ha)	No effect
N. undulata	Lab/fed infected larvae	No effect
A. bouvieri	Lab/LC50 *(Bsph)*	500X mosquito LC50
N. undulata	Lab/fed infected larvae	No effect
N. unifasciata	Field study/treated ponds	No effect
Buenoa sp.	Field study/treated ponds	No effect
Coleoptera		
Dytiscidae	Field studies	No effect
Hydrophylidae	Field studies	No effect
Crustacea		
Daphnia		
E. bampo	Laboratory *(Bti)*	100-200X mosquito rate
D. similis	Laboratory *(Bsph)*	Effect at 27,000X mosquito rate
Fairy Shrimp		
S. dichotomus	Laboratory *(Bsph)*	Effect at 15,000X mosquito rate
Crawfish		
P. clarkii	Laboratory	Effect at 1,000X mosquito rate

[1] 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. (Unless otherwise indicated, studies include evaluations of both *Bti* and *Bsph*.)

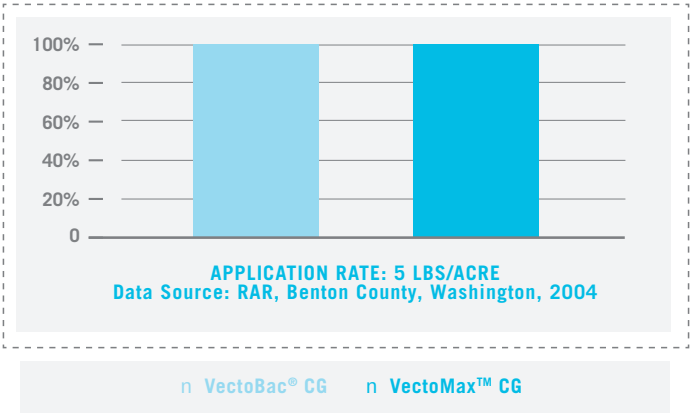
Bti and *Bsph* have been extensively tested, and they are not human health hazards when handled as instructed by the product label. VectoMax™ CG and G have a hazard classification signal word of *Caution*.

Results of VectoMax™ Granule Field Trials

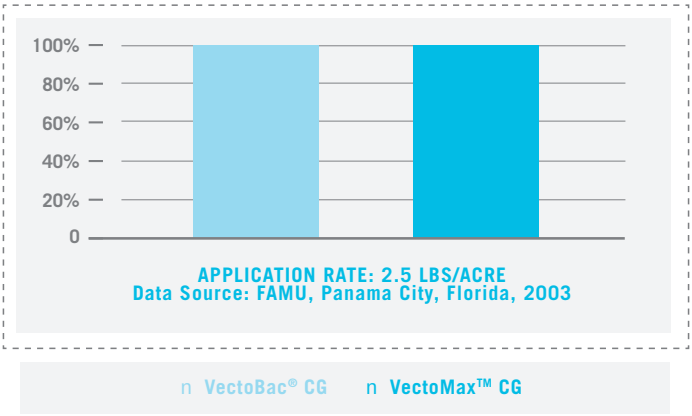
A number of small- and large-scale field trials demonstrate that VectoMax™ CG and G provide quick kill of all mosquito species in both clean and polluted waters while offering the ability to quickly assess results in the field.

QUICKLY KILLS ALL MOSQUITO SPECIES*

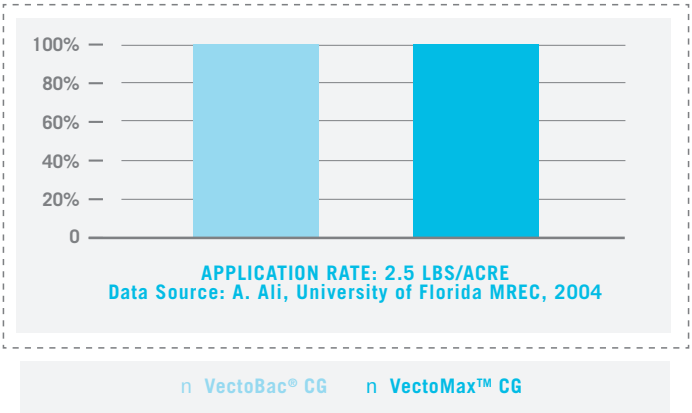
PERCENT CONTROL OF *AEDES VEXANS* IN SMALL FIELD PLOTS AT 48 HOURS POST TREATMENT



PERCENT CONTROL OF *AEDES TAENIORHYNCHUS* IN MICROCOSMS AT 48 HOURS POST TREATMENT



PERCENT CONTROL OF *ANOPHELES QUADRIMACULATUS* IN MICROCOSMS AT 48 HOURS POST TREATMENT



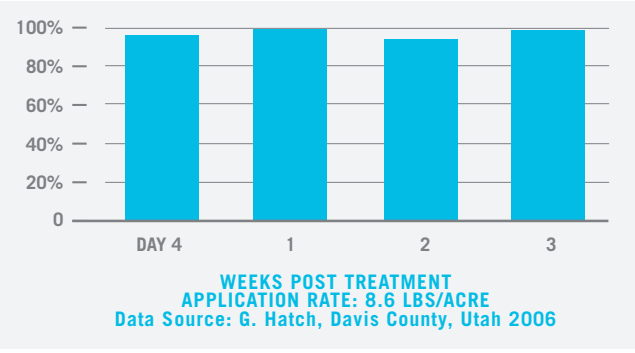
* Rates tested in the bottom two graphs (less than 5 pounds per acre) are not recommended for field applications. These studies involved microcosm test systems in which even application and consistent environmental conditions were assured. Purpose was only to demonstrate species susceptibility.

Residual Activity

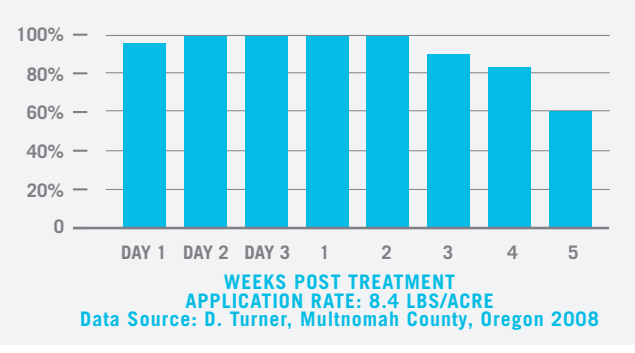
Based upon extensive field evaluations, VectoMax™ granules persist for up to 4 weeks after a single application under typical environmental conditions. Both persistence of the toxins in the water column and recycling of the bacteria contribute to the extended control.

Duration of residual control is generally determined by habitat factors rather than by application rate. Factors that can reduce residual activity include flushing and water movement, water depth, and possibly chemical contamination.

PERCENT CONTROL OF *CULEX TARSALIS* FOLLOWING AERIAL APPLICATION

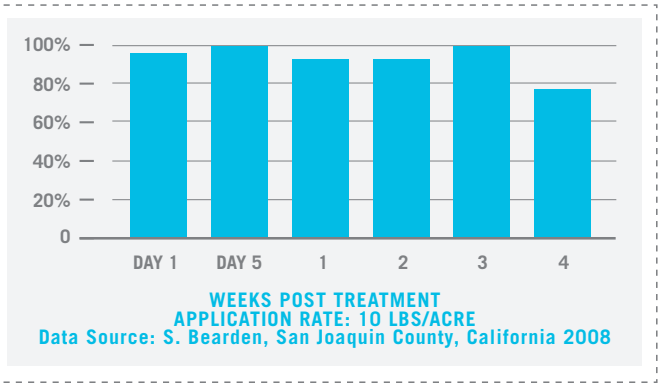


PERCENT CONTROL ON MIXED SPECIES* FOLLOWING AERIAL APPLICATION



* *Culex tarsalis, Culex pipiens*
Anopheles punctipennis, Anopheles freeborni
Culiseta incidens, Culiseta inornata

PERCENT CONTROL OF *OCHLEROTATUS MELANIMON*, *CULEX TARSALIS* AND *ANOPHELES* SPP. FOLLOWING AERIAL APPLICATION



VALENT BIOSCIENCES CORPORATION
870 Technology Way / Libertyville, IL 60048

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