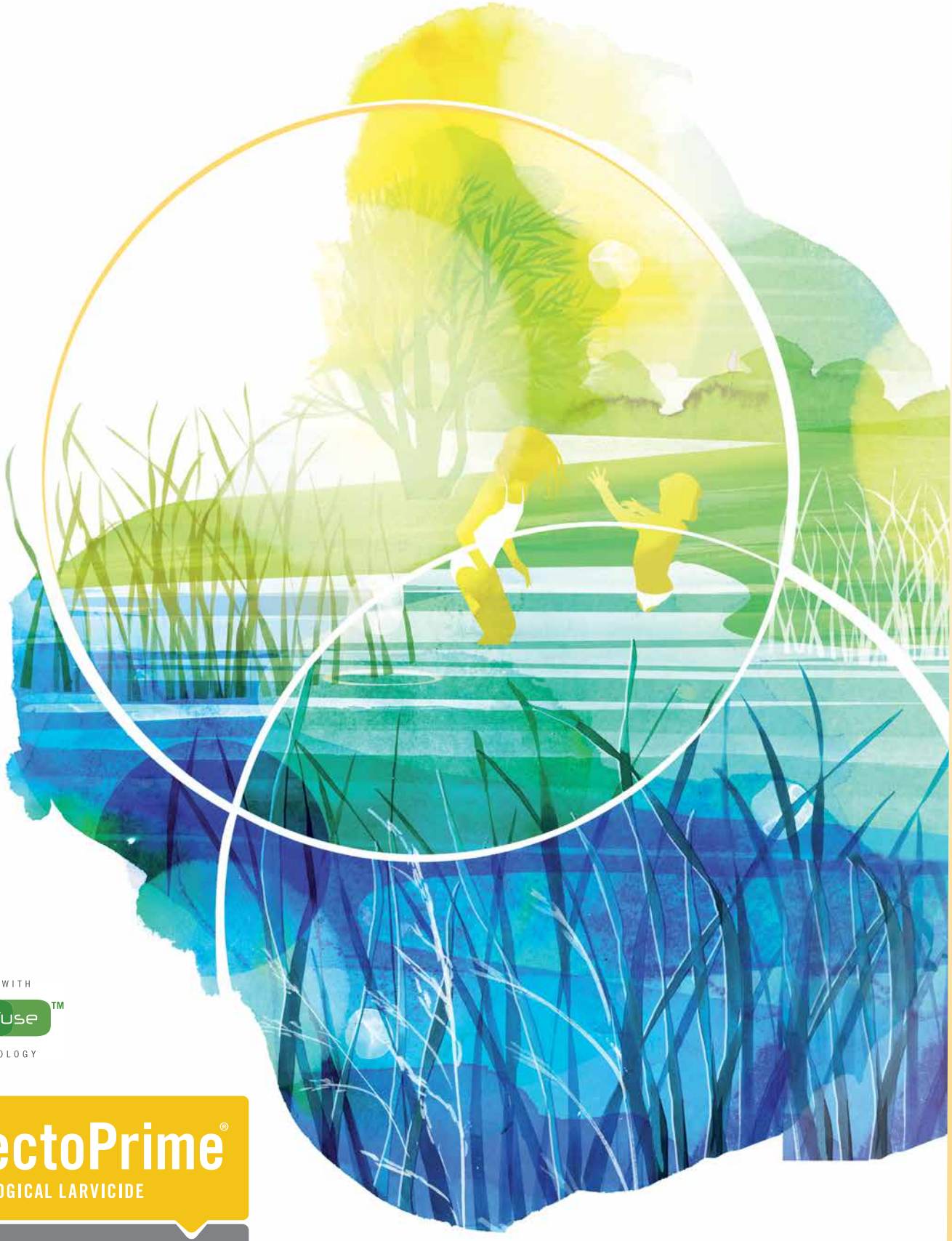


MADE WITH BioFuse™ TECHNOLOGY

VectoPrime®

BIOLOGICAL LARVICIDE

Complete Single-Brood Control

TECHNICAL USE BULLETIN

SUMITOMO CHEMICAL
AgroSolutions Division

The prime solution for complete single-brood control

VectoPrime®

MADE WITH BioFuse™ TECHNOLOGY

Single-brood/floodwater mosquitoes present two complex challenges for abatement districts. First, larvae often hatch and develop at different times and rates, which complicates strategies for treatment timing and larvicide product selection. Second, floodwater habitats can be extremely unpredictable. Flooding patterns can vary greatly based on rain patterns, tidal events, irrigation timing, etc. Consequently, labor-intensive field surveillance is often necessary to identify flooded habitats and dominant life stages present (i.e. from 1st to 4th instar) before larviciding can begin.

In order to address these challenges, Valent BioSciences developed **VectoPrime® Biological Larvicide**, a next-generation biorational larvicide for complete single-brood control that can also be applied pre-flood. VectoPrime combines *Bti* strain AM65-52 with (S)-methoprene in each micro particle by utilising Valent BioSciences BioFuse™ technology. This allows VectoPrime to offer the industry's widest single-brood application window (1st to 4th instar, or pre-flood) with the industry's lowest rates for direct application to water, thus saving significant operational costs while improving application flexibility.



Features and benefits

FEATURES	BENEFITS
Biorational larvicide with highly specific activity against mosquitoes	• Not harmful to non-target populations
BioFuse technology (combines the dual modes of action from *Bti* strain AM65-52 and (S)-methoprene into a carefully selected ratio in every micro particle)	• Can treat all larval stages • Wider application window for early or late instars • Reduces number and volume of products needed; more warehouse space
Quickly kills mosquito larvae	• Only mosquito growth regulator formulation where results can be observed within 24 hours in the field (eliminates need to collect pupae to determine efficacy)
Controls all mosquito species	• Application flexibility
Double "plus" potency and higher bulk density	• Improved payload and lower application rates significantly reduce operational costs
Pre-flood application capacity in select environments	• Reduces need to monitor flooding and wait for expected hatching

History

BUILDING ON MOSQUITO ABATEMENT PROGRAM INSIGHT

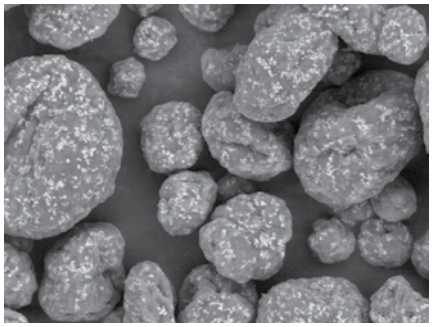
Mosquito larviciding in many countries around the globe is generally based on short-residual biorational products using one of two active ingredients: *Bacillus thuringiensis* subsp. *israelensis* or (S)-methoprene. These larvicides can provide cost-effective solutions for control of single-brood floodwater mosquitoes. However, each of these active ingredients delivers optimal activity against different mosquito larval stages. As a result, multiple products are often used to manage changing scenarios. In addition, many mosquito control programs manage multiple floodwater sites, but they have limited capacity to survey and treat all the sites once they are flooded. By the time many programs can reach a site, the larvae may have already progressed into late instars, and in some cases might have a mix of early and late instars. As a result, some programs have moved to pre-hatch products that have the residual capacity to treat multiple floods.

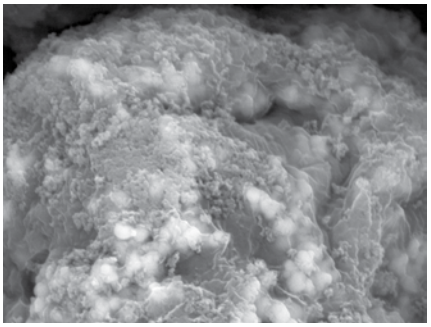
However, in many cases, residual control of multiple floods is not needed. In these cases, a single, cost effective pre-flood application is sufficient to allow program managers the "breathing room" to focus on more immediate issues. The need for single-brood product flexibility – and the desire of programs to possess dual mode of action solutions – inspired Valent BioSciences LLC to develop a next-generation tool for managing these public health problems. Valent BioSciences' experience in developing value-added products with combinations of active ingredients via its proprietary BioFuse technology led to the industry's most flexible mosquito larval control solution for single-brood mosquitoes: **VectoPrime**.

VectoPrime®
BIOLOGICAL LARVICIDE

BioFuse™ Technology

Valent BioSciences LLC's BioFuse technology combines *Bti* strain AM65-52 and (S)-methoprene in a specific toxin ratio into every micro particle of VectoPrime. This technology offers mosquito control professionals the ability to take advantage of each active ingredient's strengths, while significantly reducing the limitations that each possesses.Single-brood *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) products are most effective against early to mid instar larvae, and (S)-methoprene single-brood products are most effective against mid to late instar larvae (Fig 1). In either case, the most appropriate single-brood active ingredient to be used can be determined only after the habitat is sampled.





BioFuse particles shown using scanning electron microscope

Differences in LC50s based on active ingredient and larval instar*:

FIG.1 *Aedes aegypti*

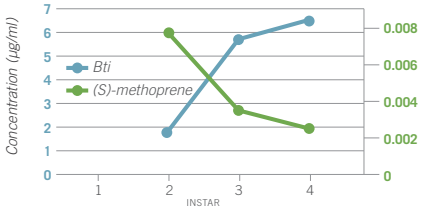
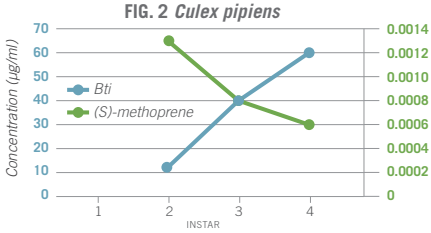


FIG. 2 *Culex pipiens*



** All figures extrapolated from the following published literature:*

***Fig 1, 2**: Phoochevin et al. 2008. Effects of cyromazin and methoprene on the developmental stages of Anopheles sirus, Aedes aegypti and Culex quinqefasciatus (Diptera: Culicidae).*

***Fig 1**: Lacey et al. 1988. Host range and selected factors influencing the mosquito larvicidal activity of the PG-14 isolate of Bacillus thuringiensis var. morrisoni. J Am Mosq Control Assoc. 1988 Mar; 4(1): 39-43.*

***Fig 2**: Boeckx et al. 2008. Communications in agricultural and applied biological sciences. Vol 73(3) 349-666.*

Efficacy

By combining *Bti* and (S)-methoprene in a single formulation, VectoPrime delivers effective control for multiple target mosquito species throughout all four instars. BioFuse technology ensures that each micro particle contains both Ais and they are delivered in appropriate ratios for consistent application and results.

Micro particles are quickly released on contact with water.

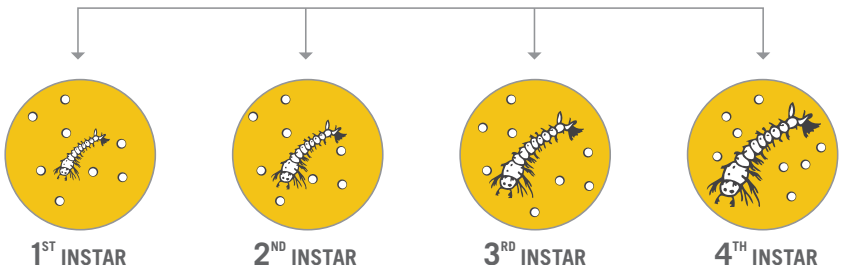
Quick kills deliver visible proof that larvae are dead.

Low application rates due to high potency and dual action.

Dual action provides complete control of asynchronous broods.

Easy to use – optimised for existing equipment and application strategies.

VectoPrime delivers effective concentration of Ais throughout all four instars



Mode of Action

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

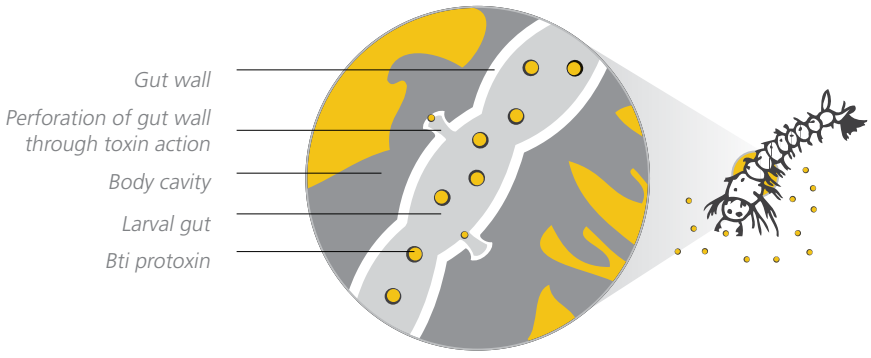
Bti is a naturally occurring spore-forming bacterium found in soil and aquatic environments throughout the world. For more than 30 years, *Bti* has played a pivotal role in public health programs by helping to control vector and nuisance insects around the world. Discovered and isolated in the 1970s, *Bti* is proven to be an effective larvicide in the fight against mosquitoes while avoiding harm to non-target populations and the environment.

At the time of sporulation, *Bti* produces complex, highly specific insecticidal crystal proteins known as protoxins. While essentially nontoxic to nearly all forms of life, when these proteins are applied to larval habitats of mosquitoes, the mosquito larvae ingest them by filter feeding. The crystal proteins are solubilised 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.

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)**

Enlarged section of midgut



While studies in a laboratory setting have shown resistance potential when individual toxins were isolated[1] from a particular strain of *Bti*[2], 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[3]. 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.

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[4]. *Bti* has been considered safe for use in aquatic environments, including drinking water reservoirs, for the control of mosquito, black fly and other closely related fly larvae[5].

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.

3 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.

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

5 Lacey LA and Merritt RW. 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.

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Mode of Action (continued)

(S)-METHOPRENE

the mid-1970s, (S)-methoprene became the first successful biorational mosquito larvicide to be used in the U.S. Its excellent safety record, very low mammalian toxicity (LD50 for oral toxicity is more than 34,000 mg/kg), and negligible long-term effects against almost all non-target populations at field rates (USEPA 2001)[6] resulted in (S)-methoprene-based products becoming a key tool for many mosquito abatement programs across the U.S.

(S)-methoprene is an insect growth regulator that acts as a mimic of the natural juvenile hormone (JH) in mosquitoes. In nature, the JH works together with the molting hormone to determine the outcome of each molt (i.e. growth stage). High concentrations of JH are needed for immature larvae to grow into larger larval stages (instars). Low concentrations of JH are required during the last larval stage (4th instar) prior to the larval-to-pupal molt, and the JH must be essentially zero in the pupae before metamorphosis can occur.

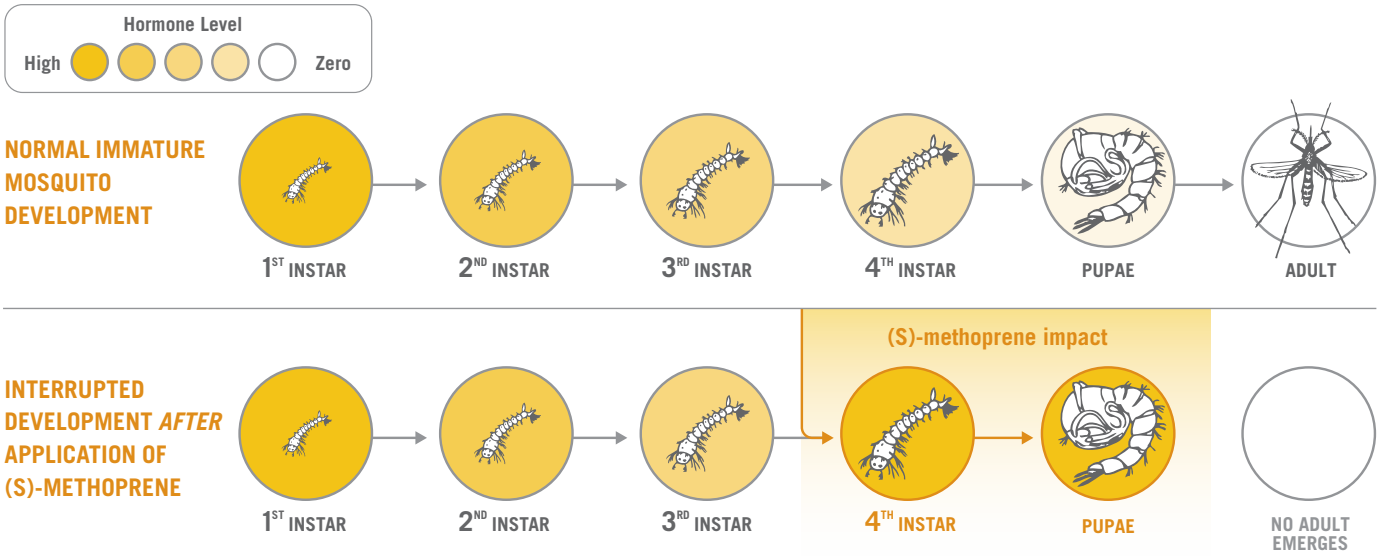
Since JH levels must be extremely low during the late 4th instar for pupation and normal adult emergence to occur, applications (or presence) of (S)-methoprene during the sensitive late 4th instar stage produce morphological abnormalities in the mosquitoes that, in most cases, lead to death during or after metamorphosis. Early instar larvae that are exposed to (S)-methoprene develop normally until they reach the pupal stage. (S)-methoprene has little effect on mosquitoes that have already reached the pupal or adult stage. Species belonging to the genera *Aedes*, *Ochlerotatus*, and *Anopheles* are the most susceptible to (S)-methoprene, whereas Culex species are less sensitive (Jakob 1972, Staal 1975)[7,8].

6 U.S. Environmental Protection Agency. 2001. Update of the March 1991 methoprene R.E.D. fact sheet. Available from U.S. Environmental Protection Agency, Washington, DC. http://www.epa.gov/pesticides/biopesticides/ingredients/factsheets/factsheet_105401.pdf.

7 Jakob WL. 1972. Additional studies with juvenile hormone-type compounds against mosquito larvae. Mosq News 32:592-595.

8 Staal GB. 1975. Insect growth regulators with juvenile hormone activity. Ann Rev Entomol 20:417-460.

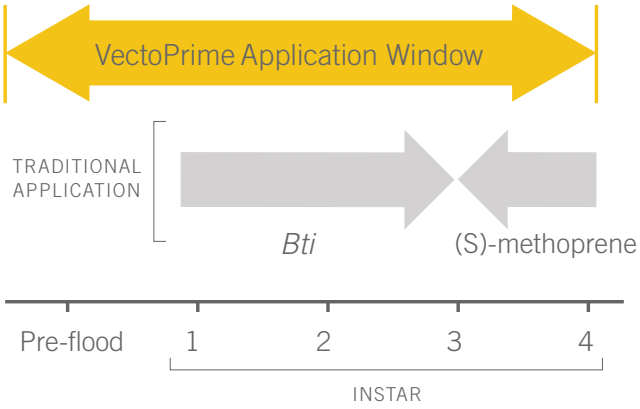
Juvenile Hormone (JH) level intensity



Flexibility

VectoPrime offers abatement professionals superior operational flexibility with a wider application window across all instars and pre-flood. The ability to apply VectoPrime prior to flooding means you can plan ahead instead of having to react to changing environmental conditions, saving time and freeing up resources that would have been required for additional field surveillance and additional treatments. In addition, working with a single solution that kills all larval instars also allows you to streamline inventories and simplify replenishment. In other words, you can start and end your week with the same solution.

Application for single brood



Efficiency

With its revolutionary double "plus" *Bti* potency formulation and higher bulk density, VectoPrime can cut application rates in half for direct applications to water compared to current single-brood products, delivering more value per hectare than conventional single-brood options. By doubling the effectiveness of every payload, you can save on fuel, maintenance, and labor costs.

The industry-standard granule size (same size as VectoBac® G and VectoLex®) streamlines calibration and characterisation of application equipment. And the unique combination of AIs means you need only perform calibration and characterisation once for your single-brood habitats. In addition, VectoPrime granules are non-abrasive, reducing attrition to application equipment when compared to sand-based products.



VectoBac G



VectoLex



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For further information on
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