Assessment of a Direct Application of Two *Bacillus thuringiensis israelensis* Formulations for Immediate and Residual Control of *Aedes albopictus*

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Source: Journal of the American Mosquito Control Association, 29(4):385-388. 2013.
Published By: The American Mosquito Control Association
DOI: http://dx.doi.org/10.2987/13-6332.1
URL: http://www.bioone.org/doi/full/10.2987/13-6332.1

Journal of the American Mosquito Control Association, 29(4):385–388, 2013

SCIENTIFIC NOTE

ASSESSMENT OF A DIRECT APPLICATION OF TWO *BACILLUS THURINGIENSIS ISRAELENSIS* FORMULATIONS FOR IMMEDIATE AND RESIDUAL CONTROL OF *AEDES ALBOPICTUS*

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ABSTRACT. The bacterial agent *Bacillus thuringiensis israelensis* (*Bti*) is a highly effective larvicide against various medically important mosquito and black fly vector species. Recent formulations of this powerful larvicidal tool have been evaluated for their field efficacy in integrated mosquito management programs. Laboratory and controlled-condition trials have indicated long periods of residual efficacy, whereas field persistence is often much lower in duration. We investigated the residual persistence of high doses of 2 formulations of *Bti*, a water-dispersible granule (VectoBac® WDG; 16 mg/liter) and an extruded pellet (VBC-60066; 80 mg/liter), for the management of natural larval populations of *Aedes albopictus*. Laboratory tests demonstrated 100% (WDG) and ≥99.7% (VBC) average mortality across all treatments over 180 days. Field tests exhibited 100% efficacy (WDG and VBC) for 3 wk against *Ae. albopictus* and other coinhabiting mosquito species, with some residual efficacy lasting for >4 wk. These results are discussed in relation to current field control of domestic *Aedes* vectors of public health significance.

KEY WORDS *Bacillus thuringiensis israelensis*, larvicide, Asian tiger mosquito, VectoBac WDG, megadose

The larvicidal control agent *Bacillus thuringiensis israelensis* de Barjac (*Bti*) has been one of the main components of integrated mosquito management programs for the past 2 decades in many parts of the world (Lacey 2007). Population suppression of mosquito vectors using biorational or chemical insecticides is often the only practicable means of large-scale disease prevention for certain emerging and significant vector-borne public health threats such as dengue and chikungunya viruses (WHO 2008, 2009). *Bacillus thuringiensis israelensis* is highly target-specific for culicid, chironomid, and simuliid larvae and has been shown to have minimal nontarget effects under field conditions (Lacey 2007, Caquet et al. 2011). The larvicidal activity of *Bti* is caused by 4 main toxins that are activated in the larval midgut, leading to cell lysis and death. This combination has so far defied substantial development of resistance despite extended periods of exposure to field populations (Lacey 2007).

Aedes albopictus (Skuse) is a highly invasive mosquito species that has become established in temperate regions of the USA, and continues to expand its range (Farajollahi and Nelder 2009, Farajollahi and Price 2013, Rochlin et al. 2013). A 2007 epidemic of chikungunya virus was solely driven by *Ae. albopictus* populations (Tsetsarkin et al. 2007). The public health importance of *Ae. albopictus* in urban and suburban areas where disease transmission to humans is of concern makes suppression of this species a priority for vector control programs (Unlu et al. 2011, Fonseca et al. 2013). The removal, draining, or treatment of all potential container larval habitats in peridomestic areas is extremely labor intensive and requires constant attention (Bartlett-Healy et al. 2011). Consequently, the preferred larvicide for control of a container-inhabiting mosquito, such as *Ae. albopictus*, should be one that provides extended residual activity.

Bacillus thuringiensis israelensis larvicidal activity has been known to be influenced by prevailing biotic and abiotic environmental conditions acting on larval habitats (Lacey 2007). Such variables that can affect *Bti* efficacy include temperature, ultraviolet or sunlight exposure, container type, treatment dosage, water renewal, organic content, target mosquito species, larval stage, feeding behavior, and larval density (Nayar et al. 1999). While *Bti* is conventionally considered a single-brood application tool with short field persistence, simulated field studies in shaded conditions with covered containers have indicated longer residual activities of 3–4 months (Mulla et al. 2004) and 5–6 months (Benjamin et al. 2005). However, investigations under typical field conditions have found limited residual persistence of only 2 days (Toma et al. 2003) and 1–4 wk (Batra et al. 2000). Efficacy of a water-dispersible granular formula-

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tion of *Bti*, VectoBac® WDG, has been shown against *Aedes aegypti* (L.) for 3 months, and though pesticide efficacy was reduced to 3 wk under sunlight exposure (Vilarinhos and Monnerat 2004), it would appear that formulation type is the key means to achieve extended persistence.

Ritchie et al. (2010) determined that residual efficacy of particularly high doses (''megadoses'') of WDG can provide 2–6 months of larvicidal suppression from a single application against *Ae. aegypti* exposed to natural temperature fluctuations. However, the results may have been affected by protection from water renewal by a cloth mesh over the outer container and also from sunlight exposure (limited ultraviolet degradation), as well as absence of natural continual oviposition and larval competition pressures that would be affected under normal field use. Here we investigate the efficacy and residual activity of a high-dose application (2× the maximum recommended concentration) of 2 *Bti* formulations on colonized and natural populations of *Ae. albopictus*.

The formulations of *Bti* strain AM 65-52 utilized were the water-dispersible granular VectoBac WDG and an extruded pellet VBC-60066 (Valent BioSciences, Libertyville, IL). WDG has a potency of 3,000 international toxic units (ITU) per milligram, while VBC has a potency of 800 ITU/mg. Recommended application rates for WDG are 8 mg/liter and for VBC 40 mg/liter (P. DeChant, Valent BioSciences, personal communication). The WDG formulations mix readily with water, and direct application to artificial containers quickly spreads across the surface and suspends in the water column before settling and ingestion by mosquito larvae (Su and Mulla 1999).

Second-stage *Ae. albopictus* larvae utilized in the indoor efficacy trials were obtained from a laboratory colony recently isolated from endemic populations in Mercer County, NJ, as described previously (Nelder et al. 2010). Field trials utilized natural endemic mosquito populations within the experimental site.

Laboratory trials were conducted in translucent 473-ml polyethylene terephthalate cups (Solo Cup Company, Lake Forrest, IL) in replicates of 5 for each treatment condition of WDG at rates of 0, 8, 16, and 32 mg/liter and VBC at rates of 0, 40, 80, and 160 mg/liter. Each cup held 245 ml of tap water, 5 ml of bacterial inoculum (filtered local natural larval habitat), and 1 g of crushed red oak (*Quercus rubra* L.) leaves. After a 4-day acclimation period, 10 larvae were added. The following actions were taken at 4-day intervals for over a 180-day test period: larval mortality was monitored, evaporated water was replenished, all larvae were removed (alive or dead), cup location was rotated within the incubator, and 10 new 2nd-stage *Ae. albopictus* from the laboratory colony were added to each cup. The study was conducted at 25°C, 50% RH, and a 12-hour light to 12-hour dark photoperiod.

Field trials were conducted from August 14 to November 6, 2009, at an abandoned industrial site in Trenton, NJ. This site harbored large numbers of discarded artificial containers (primarily automobile tires) and provided adequate vegetation and shade to sustain large populations of *Ae. albopictus*. Eleven-liter black polyethylene buckets with holes drilled at the 8-liter mark were randomly placed in shade ≥20 m apart. Ten replicates each of 8-liter dechlorinated tap water and 5 g of crushed oak leaves were acclimated for 2 wk prior to the initial pretreatment sampling and direct (neat) application of WDG (16 mg/liter), VBC (80 mg/liter), and control treatments. Larval sampling was conducted every 4 days for 80 days following the treatment using an aquarium net (25 × 18 cm), following sampling protocols by Nelder et al. (2010). Collected mosquitoes were brought to the laboratory for enumeration and species identification. Water was added as necessary to maintain a consistent 8-liter volume after each sampling.

Statistical analyses for field trials were performed using a REPEATED statement in PROC MIXED (SAS Institute, Cary, NC). Independent variables were treatment, time, and their interaction. Response variables for the field trials were abundances (log transformed). Least square estimates were obtained and compared for each period using Tukey's adjustment.

Laboratory trials displayed an average mortality of >99.7% across all treatments for the 180-day study period. All 3 WDG rates provided 100% larval mortality. VBC provided 100%, 99.9%, and 99.7% average mortality at the 160 mg/liter, 80 mg/liter, and 40 mg/liter application rates. Ten larvae (0.1%) survived the VBC exposure: 7 individuals at the 40 mg/liter rate, 2 at 80 mg/liter, and 1 at 160 mg/liter. Control mortality averaged 1.8%.

Pretreatment collections from the buckets included 8 species (2,849 specimens): *Culex pipiens* L. (40%), *Ae. albopictus* (28%), *Cx. restuans* Theobald (17%), *Ae. japonicus* (Theobald) (13%), *Anopheles punctipennis* (Say) (1%), *Ae. atropalpus* (Coquillett), *Toxorhynchites rutilus septentrionalis* (Dyar and Knab), and *Ae. triseriatus* (Say). Natural infestation in control buckets (posttreatment) were: *Ae. albopictus* (34%), *Cx. restuans* (25%), *Cx. pipiens* (21%), and *Ae. japonicus* (18%).

There was a significant interaction between treatment and time for both *Ae. albopictus* and all other species combined, indicating that the slopes were not equal (*Ae. albopictus*: df = 2, F = 10.72, $P = <0.0001$. All other species: df = 2, F = 24.96, $P = <0.0001$). The slope for control was significantly different from VBC and WDG until the 48th day (Fig. 1). The WDG provided 100% suppression of *Ae. albopictus* for 3 wk (Figs. 1A and 2A). The VBC provided 100% suppression of all mosquito species throughout, apart from 2 *Ae.*

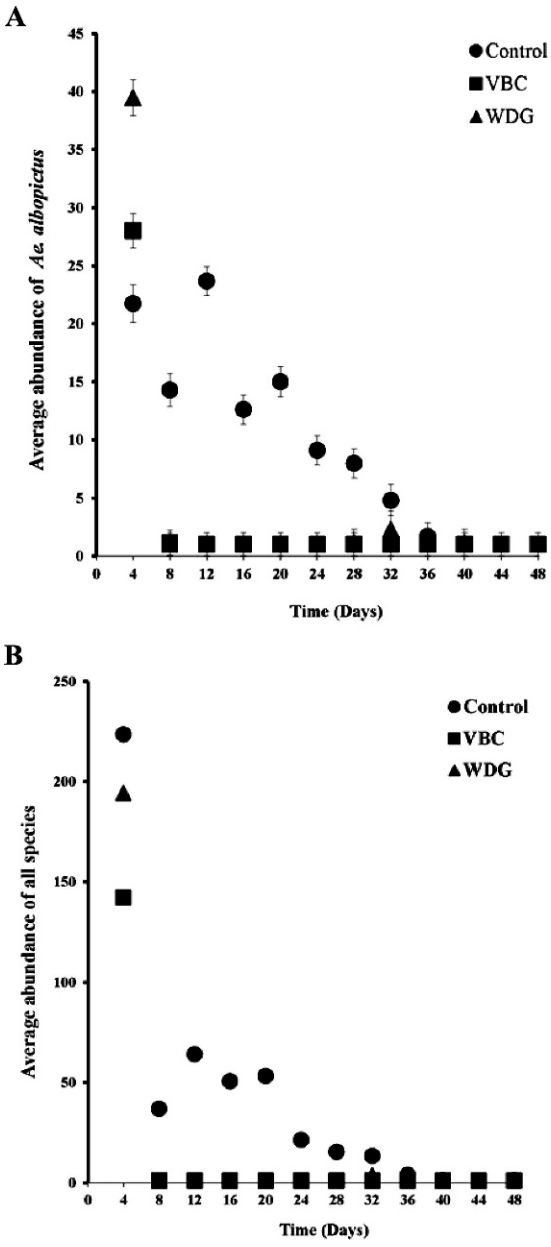

Fig. 1. Back-transformed (log average abundance) mean ± SE of (A) *Aedes albopictus* and (B) all larval species from each treatment type of VectoBac® WDG, VBC, and control bucket habitats.

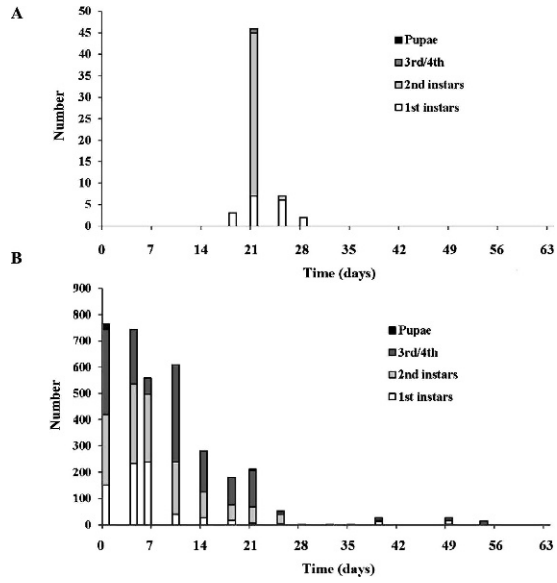

Fig. 2. Posttreatment stacked distribution of larval developmental stages of *Aedes albopictus* from the field trial treatments (A) VectoBac® WDG and (B) controls.

albopictus pupae encountered on the 1st posttreatment sampling. Control collections provided a mixture of all instars (Fig. 2B), and treatment populations never reached the 3rd- or 4th-stage larval developmental stage, apart from a single anopheline larva (Fig. 2A). Laboratory trials yielded >99% larval mortality over the test period for both formulations at all dosages. But larvicide efficacy evaluations are most informative under conditions encountered naturally, such as in open-container field trials.

Our open-container field trials revealed 100% residual activity of WDG and VBC for at least 21 days against all developmental larval stages. Previously, a 2-day residual per application was found in public parks in Italy for a tablet formulation of *Bti* (Toma et al. 2003). A natural infestation field trial in Malaysia using the same formulation on both *Ae. albopictus* and *Ae. aegypti* provided 100% residual efficacy for 6 days in earthen containers, 12 days in plastic containers, and 19 days for high-density polyethylene containers (Benjamin et al. 2005).

Our 1st field sampling conducted was in September, and the study continued until November when natural mosquito populations were declining or nonexistent. Consequently, the full efficacy of the products from the beginning of the season remains to be determined.

The WDG and VBC provided 100% suppression of pupae and 3rd/4th instars, with 2 notable exceptions. First, 2 pupae from initial posttreatment VBC collection, is explained by the mode of action of *Bti*, via uptake into the larval midgut through grazing and filter-feeding, since pupae do not feed. The other, a single *Anopheles* pupa within WDG buckets, is not unexpected as *Anopheles* feed at the surface and would not be exposed as much to *Bti* formulations intended for *Aedes* (and *Culex*) species, designed to settle at their trophic zone at the bottom and sides of containers (Aly et al. 1987).

The WDG and VBC formulations of *Bti* were effective against natural *Ae. albopictus* popula-

tions for at least 3–4 wk at 16 mg/liter and 80 mg/liter, respectively. The WDG formulation has been in regular use as part of large-scale integrated mosquito management strategy for container-inhabiting mosquitoes in the dengue control program in Brazil since 2006. The results of our field study corroborate the use of these *Bti* formulations as part of an overall mosquito management strategy against *Ae. albopictus* populations. Natural infestation evaluations of "megadose" concentrations may be of particular interest in future studies to perhaps increase field residual persistence (Ritchie et al. 2010) and afford public health officials a much needed and enduring mosquito management tool to combat a peridomestic disease vector such as *Ae. albopictus*.

We thank Anthony Herold, Ian O'Neill, and Heather Evans for field and laboratory assistance. We thank Peter DeChant and Jim Andrews for valuable input and discussions regarding our field trials and biorational pesticides. We also thank the anonymous reviewers for comments on the original submission of this paper.

REFERENCES CITED

Aly C, Mulla MS, Schnetter W, Xu B. 1987. Floating bait formulations increase effectiveness of *Bacillus thuringiensis* var. *israelensis* against *Anopheles* larvae. *J Am Mosq Control Assoc* 3:583–588.

Bartlett-Healy K, Hamilton G, Healy SP, Crepeau T, Unlu I, Farajollahi A, Fonseca DM, Gaugler R, Clark GC, Strickman D. 2011. Source reduction behavior as an independent measurement of the impact of a public health education campaign in an integrated vector management program for the Asian tiger mosquito. *Int J Environ Res Public Health* 8:1358–1367.

Batra CP, Mittal PK, Adak T. 2000. Control of *Aedes aegypti* breeding in desert coolers and tires by use of *Bacillus thuringiensis* var. *israelensis* formulation. *J Am Mosq Control Assoc* 16:321–323.

Benjamin S, Rath A, Fook CY, Lim LH. 2005. Efficacy of a *Bacillus thuringiensis israelensis* tablet formulation, VectoBac DT®, for control of dengue mosquito vectors in potable water containers. *Southeast Asian J Trop Med Public Health* 36:879–892.

Caquet T, Roucaute M, Le Goff P, Lagadic L. 2011. Effects of repeated field applications of two formulations of *Bacillus thuringiensis* var. *israelensis* on non-target saltmarsh invertebrates in Atlantic coastal wetlands. *Ecotoxicol Environ Saf* 74:1122–1130.

Farajollahi A, Nelder MP. 2009. Changes in *Aedes albopictus* (Diptera: Culicidae) populations in New Jersey and implications for arbovirus transmission. *J Med Entomol* 46:1220–1224.

Farajollahi A, Price DC. 2013. A rapid identification guide for larvae of the most common North American container-inhabiting *Aedes* species of medical importance. *J Am Mosq Control Assoc* 29:203–221.

Fonseca DM, Unlu I, Crepeau T, Farajollahi A, Healy SP, Bartlett-Healy K, Strickman D, Gaugler R, Hamilton G, Kline D, Clark GG. 2013. Area-wide management of *Aedes albopictus*: II. Gauging the efficacy of traditional integrated pest control measures against urban container mosquitoes. *Pest Manag Sci* (in press) doi: 10.1002/ps.3511.

Lacey LA. 2007. *Bacillus thuringiensis* serovariety *israelensis* and *Bacillus sphaericus* for mosquito control. *J Am Mosq Control Assoc* 23:133–163.

Mulla MS, Thavara U, Tawatsin A, Chompoosri J. 2004. Procedures for the evaluation of field efficacy of slow-release formulations of larvicides against *Aedes aegypti* in water-storage containers. *J Am Mosq Control Assoc* 20:64–73.

Nayar JK, Knight JW, Ali A, Carlson DB, O-Bryan PD. 1999. Laboratory evaluation of biotic and abiotic factors that may influence larvicidal activity of *Bacillus thuringiensis* serovar. *israelensis* against two Florida mosquito species. *J Am Mosq Control Assoc* 15:32–42.

Nelder M, Kesavaraju B, Farajollahi A, Healy S, Unlu I, Crepeau T, Ragavendran A, Fonseca D, Gaugler R. 2010. Suppressing *Aedes albopictus*, an emerging vector of dengue and chikungunya viruses, by a novel combination of a monomolecular film and an insect-growth regulator. *Am J Trop Med Hyg* 82:831–837.

Ritchie SA, Rapley LP, Benjamin S. 2010. *Bacillus thuringiensis* var. *israelensis* (Bti) provides residual control of *Aedes aegypti* in small containers. *Am J Trop Med Hyg* 82:1053–1059.

Rochlin I, Ninivaggi DV, Hutchinson ML, Farajollahi A. 2013. Climate change and range expansion of the Asian tiger mosquito (*Aedes albopictus*) in northeastern USA: implications for public health practitioners. *PLoS One* 8:e60874.

Su T, Mulla MS. 1999. Field evaluation of new water-dispersible granular formulations of *Bacillus thuringiensis* spp. *israelensis* and *Bacillus sphaericus* against *Culex* mosquitoes in microcosms. *J Am Mosq Control Assoc* 27:356–365.

Toma L, Severini F, Bella A, Romi R. 2003. A semifield evaluation of VectoBac® DT (ABG-6499), a new formulation of *Bacillus thuringiensis israelensis* for control of *Aedes albopictus*. *J Am Mosq Control Assoc* 19:424–429.

Tsetsarkin KA, Vanlandingham DL, McGee CE, Higgs S. 2007. A single mutation in chikungunya virus affects vector specificity and epidemic potential. *PLoS Pathog* 3:e201.

Unlu I, Farajollahi A, Healy SP, Crepeau T, Bartlett-Healy K, Williges E, Strickman D, Clark GC, Gaugler R, Fonseca DM. 2011. Area-wide management of *Aedes albopictus*: choice of study sites based on geospatial characteristics, socioeconomic factors, and mosquito populations. *Pest Manag Sci* 67:965–974.

Vilarinhos PTR, Monnerat R. 2004. Larvicidal persistence of formulations of *Bacillus thuringiensis* var. *israelensis* to control larval *Aedes aegypti*. *J Am Mosq Control Assoc* 20:311–314.

WHO [World Health Organization]. 2008. *Chikungunya fact sheet 327, March 2008* [Internet]. Geneva, Switzerland: World Health Organization [accessed June 3, 2011]. Available from: http://www.who.int/mediacentre/factsheets/fs327/en/.

WHO [World Health Organization]. 2009. *Dengue guidelines for diagnosis, treatment, prevention and control.* WHO/HTM/NTD/DEN/2009.1. Geneva, Switzerland: World Health Organization.