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EFFECTIVENESS OF AERIAL- AND GROUND-APPLIED *BACILLUS* FORMULATIONS AGAINST *ANOPHELES QUADRIMACULATUS* LARVAE IN ARKANSAS RICE PLOTS[1,2]

JAMES A. DENNETT AND MAX V. MEISCH

Department of Entomology, University of Arkansas, Fayetteville, AR 72701

ABSTRACT. Experimental *Bacillus* larvicides designed to float on or near the water surface were compared to labeled standard *Bacillus* corn-cob–based larvicides using sentinel *Anopheles quadrimaculatus* larvae in Arkansas rice plots during the 1998 growing season. Experimental floating formulations of *Bacillus thuringiensis israelensis* applied at 5.58 and 11.18 kg/ha provided up to 100% control of 3rd- and 4th-stage *Anopheles* larvae within 24–48 h, whereas the water-dispersible granule formulations containing *Bacillus sphaericus* required 48–72 h to yield >75% mortality in 0.16-ha plots at 11.18 kg/ha. Detecting and targeting the smaller developmental stages (1st- and 2nd-stage larvae) could increase the effectiveness of the tested compounds against *An. quadrimaculatus* in Arkansas and other rice-growing regions.

KEY WORDS *Anopheles quadrimaculatus, Bacillus thuringiensis israelensis, Bacillus sphaericus,* VectoBac®, VectoLex®, water-dispersible granule

INTRODUCTION

Conventional biorational larvicides containing *Bacillus thuringiensis israelensis* de Barjac and *Bacillus sphaericus* Neide have limited effectiveness against *Anopheles quadrimaculatus* Say because larvae feed primarily at the water surface. Feeding behavior, body positioning, and ingestion rates of *Anopheles* larvae at the water surface have been thoroughly documented (Rashed and Mulla 1989, Merritt et al. 1992). Bacteria and particulate matter up to 50 μm in size within the surface microlayer (<1 mm) permit growth of *Anopheles* larvae because of presence of an abundant source of food (Wallace and Merritt 1980, Walker and Merritt 1993, Wotton et al. 1997). Summarily, *Anopheles* larvae feed slowly at the water surface on very small particles for long periods of time compared to other genera. In addition to having different feeding behavior from other mosquito genera, *Anopheles* larvae also differ from both *Aedes* and *Culex* in that *Anopheles* consume food particles from the air–water interface at filtration rates 10–20 times smaller than either *Aedes* or *Culex,* resulting in lower rates of ingested *B. thuringiensis* Berliner toxin (Aly et al. 1987). Although surface feeding predominates, Dahl et al. (1988) stated that under laboratory conditions, *Anopheles gambiae* Giles had some tendency towards brushing behavior on substrates as well. While rearing *An. quadrimaculatus* larvae for tests performed during the summer of 1998, this behavior was observed on numerous occasions with 3rd-and 4th-stage *An. quadrimaculatus* larvae grazing the substrate in the bottom of rearing trays. Dahl et al. (1988) stated that this flexibility of feeding behavior constitutes a major challenge when considering control measures.

The objectives of this research were to compare experimental *Bacillus* formulations designed to float on or near the water surface with labeled corn-cob–based formulations of *Bacillus* using sentinel *An. quadrimaculatus* larvae in Arkansas rice plots during the 1998 growing season.

MATERIALS AND METHODS

Rice plots: All tests were performed at the University of Arkansas Rice Research and Extension Center located near Stuttgart, AR, during July 1998. Eighteen leveed plots measuring 18.2 × 91.4 m (0.41acre or 0.16 ha) were used in the aerial application portion of these field tests. Forty-five smaller plots, each measuring 7.9 × 7.9 m (676 ft^2 or 62.8 m^2), were used for tests requiring ground application. All plots were separated by earthen levees. The water depths in large and small plots were 20.3 cm (8 in.) and 10.1 cm (4 in.), respectively. All plots were drill-seeded with 'Cypress' variety rice and flooded about 1 month before testing, with plant height approaching 60.9 cm (24 in.). Water levels were maintained throughout the summer and closely monitored to prevent cross-contamination due to overflooding.

Mosquitoes: In the past, 2 of 5 sibling species within the *An. quadrimaculatus* Complex have been recorded in Arkansas County, but they are associated with different habitats. *Anopheles quadrimaculatus* is associated primarily with flooded rice, whereas *Anopheles smaragdinus* Reinert is found in permanent water swamps (Reinert et al. 1997). Eggs were harvested from *An. quadrimaculatus* females aspirated from livestock barns bordered by large rice fields, and subsequent larvae were mass-reared (Dennett and Meisch, unpublished data).

[1] Mention of commercial products does not imply a recommendation for use or sale by the University of Arkansas.

[2] Approved for publication by the Director of the Arkansas Agricultural Experiment Station.

Upon attaining the proper developmental stage, groups of 10 larvae were transferred from shallow rearing pans to 236-ml unwaxed paper cartons containing water for transport to the field for installation in sentinel cages.

Sentinel cages: A total of 92 floating sentinel cages (hereafter referred to as floaters) described by Sandoski et al. (1986) were used during the course of testing in the plots. For the small plot tests, 10 late 3rd- to early 4th-stage larvae were placed within a single floater in each treatment plot for each installation date with 3 replicates per treatment. Large plots had 2 floaters, each with 10 late 3rd- to early 4th-stage larvae per cage and replicated 3 times. Each floater was subsequently covered with tulle and fastened with rubber bands to exclude predators. On each larval installation, any larvae remaining in the floaters were carefully discarded before installing fresh larvae to determine if compounds applied to the plots remained active.

Both areas with large and small plots were separately randomized in complete block designs, with 3 plots dedicated to each formulation, and 3 plots serving as untreated controls. Before insecticide application in the small plots, a single, uncovered floater was placed on the edge of the pan in each small plot so that when an application was made, the floater received a dosage of the insecticide. In the large plots, 2 floaters were positioned in the middle of the pan, each placed 30.4 m (100 ft) from either end, before aerial application. The canopy thickness required the removal of several plants to permit floater placement and subsequent relocation during the tests. When observing the floaters each day, care was taken not to dislodge the silty film at the bottom of the floaters, which could have contained active residual material. Daily readings of each floater were made regarding the number of live and dead mosquito larvae, and presence of predators.

Microbial agents: The 4 microbial larvicides used during this study consisted of VectoBac® G and VectoBac® ABG-6495 (an experimental floating formulation), each containing *B. thuringiensis* (serotype H-14, 200 ITU/mg), and VectoLex® CG and VectoLex® WDG, each containing *B. sphaericus* (serotype H5a5b, strain 2362, 50 ITU/mg [CG], and 600 ITU/mg [WDG]). Materials used in all plot treatments were applied at rates comparable to labeled rates (VectoBac G and ABG-6495 and VectoLex CG) or ITU equivalent to labeled product rates (VectoLex WDG). All materials were provided by Abbott Laboratories, North Chicago, IL.

VectoBac formulations: Two tests were performed with VectoBac formulations, 1 containing 12 small plots and 1 containing 12 large plots. The 12 randomized small plots consisting of 3 replications of 3 insecticide treatments (9 plots) each were hand-seeded with either VectoBac G at a rate of 5 lb/acre (5.58 kg/ha), ABG-6495 at a rate of 5 lb/acre (5.58 kg/ha), or ABG-6495 at a rate of 10 lb/acre (11.18 kg/ha). The remaining 3 small plots served as controls. Two installations of larvae were introduced for each plot (0, 24 h posttreatment), and were monitored up to 3 days posttreatment.

The large-plot VectoBac test methodology was generally the same as described earlier; the only exception was the aerial application. The formulations were applied using a calibrated Thrush Turbo® aircraft flying at 140 mph (225 km/h) at an altitude of 10 ft (3 m). Two passes by the aircraft were required to apply ABG-6495 at 10 lb/acre (11.18 kg/ha), because equipment was calibrated for 5 lb/acre (5.58 kg/ha). Two installations of larvae were made for each plot (0, 72 h posttreatment), which was monitored up to 6 days posttreatment.

VectoLex formulations: Three tests were performed using the VectoLex formulations, 2 small-plot tests and 1 large-plot test. The 1st consisted of 15 randomized small plots, consisting of 3 replications of 4 insecticide treatments (12 plots), with 3 additional plots serving as controls. VectoLex CG was applied at a rate of 10 lb/acre (11.18 kg/ha), whereas VectoLex WDG was applied at ITU equivalent rates corresponding to 5, 10, and 15 lb/acre (5.58, 11.18, and 16.79 kg/ha, respectively). VectoLex CG and VectoLex WDG were measured out as previously described, with VectoLex CG placed within unwaxed cartons. The WDG was measured and funneled into clean plastic 2-liter beverage containers filled with tap water and immediately capped off before transport. The CG was hand-seeded within specific small plots, whereas the WDG was applied using a CO_2 pressurized sprayer with adjustable nozzle, calibrated at 30 psi. Two installations of larvae were made for each plot (0 and 72 h) and were monitored daily for 7 days posttreatment.

The 2nd test assessed VectoLex in a large-plot test that consisted of 9 plots, that is, 3 replications of 2 insecticide treatments, plus 3 control plots. After calibration, VectoLex CG and VectoLex WDG were applied to individually designated plots using the aircraft and methodology previously described at CG rate equivalent to 10 lb/acre (11.18 kg/ha), and WDG rate equivalent to 10 gal/acre (93.48 liter/ha) with 1,135 g of WDG powder added to the hopper before filling with water. Two installations of larvae were made for each plot (0, 72 h posttreatment) and were monitored daily for 6 days posttreatment.

The 3rd VectoLex test was performed to assess the efficacy of WDG on another common rice-land mosquito species, *Psorophora columbiae* (Dyar and Knab). VectoLex CG and VectoLex WDG were applied at the 11.18 kg/ha equivalent to 3 plots each with 3 additional plots serving as controls. Two floaters were assigned to each plot, that is, 1 floater containing 10 *Anopheles* larvae as described earlier, and 10 late 3rd- to early 4th-stage wild-caught *Psorophora* larvae installed in the 2nd floater. Two larval installations were made for each species in each

Table 1. Mean percent mortality of *Anopheles quadrimaculatus* larvae in rice plots treated with standard and experimental formulations of *Bacillus thuringiensis* and *Bacillus sphaericus*.[1,2]

Formulation	Application rate (lb/acre [kg/ha])	Plot size	Days posttreatment						
			1	2	3	4	5	6	7
Larvae installation no.			1	1	1	2	2	2	
VectoBac®									
G	5 (5.58)	Large	56.2c	49.9c	59.9	61.9a	58.3a	74.9a	
ABG-6495	5 (5.58)	Large	83.3b	81.5b	74.9	61.9a	69.4a	74.9a	
ABG-6495	10 (11.18)	Large	87.5a	100.0a	—[3]	61.9a	80.5a	89.2a	
Larvae installation no.			1	2	2				
VectoBac									
G	5 (5.58)	Small	100.0a	36.8a	29.9				
ABG-6495	5 (5.58)	Small	100.0a	73.6b	79.9				
ABG-6495	10 (11.18)	Small	100.0a	100.0c	—[3]				
Larvae installation no.			1	1	1	2	2	2	2
VectoLex®									
CG	10 (11.18)	Small	77.7a	84.9a	88.2a	7.1c	11.9c	14.9c	49.9c
WDG (1×)	5 (5.58)	Small	85.1a	79.9a	100.0a	21.4a	31.9a	24.9a	49.9a
WDG (2×)	10 (11.18)	Small	81.4a	74.9a	82.3a	21.4a	39.9a	29.9a	71.3a
WDG (3×)	15 (16.79)	Small	92.5a	94.9a	100.0a	60.7ab	55.9ab	74.9ab	78.5ab
Larvae installation no.			1	1	1	2	2		
VectoLex									
CG	10 (11.18)	Large	64.2a	83.3a	84.9a	24.2	—[3]		
WDG	10 (11.18)	Large	53.5a	75.0a	89.9a	15.1	—[3]		

[1] For a specific test, on a given day posttreatment, for a specific larval installation, means followed by the same lowercase letter are not significantly different from one another ($P > 0.05$) by adjusted t-tests.
[2] Percentages are adjusted using Abbott's formula.
[3] Readings not performed because of 100% reduction of or lack of efficacy against a specific installation.

treatment plot at 0 and 72 h posttreatment and were monitored daily for up to 5 days.

Data obtained from the plot tests were corrected for control mortality and arcsine transformed before conducting an analysis of variance to test the hypothesis that for each larval installation, mean mortalities for all treatments within a particular test were equal (Abbott 1925). Separation of treatment mean mortalities was performed using the Tukey–Kramer honestly significant difference adjusted t-test (Sall and Lehman 1996).

RESULTS

VectoBac large-plot test

The high rate (11.18 kg/ha) of ABG-6495 produced a significant reduction ($P < 0.05$) of 100% at 2 days posttreatment compared to the 81.5% obtained by the same product at half the rate (Table 1). Interestingly, VectoBac G (5.58 kg/ha) did not exceed 60% control until 4 days posttreatment and slowly rose to achieve 74.9% control at 6 days posttreatment. With the 1st installation of larvae, significant differences ($P < 0.05$) were found among treatments at 24 and 48 h posttreatment. Although some residual activity for all 3 treatments was present (74.9, 74.9, and 89.2%, respectively, for VectoBac G [5.58 kg/ha] and ABG-6495 [5.58 and 11.18 kg/ha]) at 6 days posttreatment, no significant differences ($P > 0.05$) were detected among treatments of the 2nd larval installation (Table 1).

VectoBac small-plot test

The 1st installation of larvae resulted in 100% mortality in all 3 treatments at 24 h (Table 1). Significant differences in treatments ($P < 0.05$) were recorded in the 2nd installation with VectoBac G (5.58 kg/ha) showing little activity (36.8%), whereas ABG-6495 at 5.58 and 11.18 kg/ha, respectively, yielded 73.6 and 100% control at 48 h posttreatment. These results were generally consistent with mortalities in the VectoBac large-plot test. Field data collection was discontinued after 72 h, when percentage mortality dropped below 30% with VectoBac G.

VectoLex large-plot test

Both VectoLex CG and VectoLex WDG at 11.18 kg/ha rates were initially slow acting (<65% con-

Table 2. Mean percent mortality of *Anopheles quadrimaculatus* (*An.*) and *Psorophora columbiae* (*Ps.*) larvae in small rice plots treated with standard and experimental formulations of *Bacillus sphaericus*.[1]

Formulation	Application rate (lb/ac [kg/ha])	Plot size	Days posttreatment					
			1		4		5	
			An.	*Ps.*	*An.*	*Ps.*	*An.*	*Ps.*
Larvae installation no.			1	1	2	2	2	2
VectoLex CG	10 (11.18)	Small	6.8	100.0	9.9	10.0	17.5	29.1
VectoLex WDG	10 (11.18)	Small	17.1	100.0	24.9	6.6	29.3	0.0

[1] Percentages were adjusted using Abbott's formula.

trol at 24 h), but increased gradually to >84% control at 72 h. VectoLex WDG approached 90% reduction of the 1st installation (Table 1). Treatment means were not significantly different ($P > 0.05$) at 24, 48, and 72 h posttreatment. Readings for the 2nd installation were not performed past 4 days posttreatment, because percent control was low (<25%) for both compounds.

VectoLex small-plot test

During the 1st larval installation, no significant differences were found among VectoLex CG at 11.18 kg/ha and VectoLex WDG at 5.58, 11.18, and 16.79 kg/ha rates, with all formulations achieving >75% control. VectoLex WDG at 16.79 kg/ha achieved 92.5% control during the 1st 24 h posttreatment, whereas 100% control did not occur until 3 days posttreatment in plots containing VectoLex WDG at the 5.58 and 16.79 kg/ha rates (Table 1). Significant differences were found in the 2nd larval installation, with VectoLex CG at 11.18 kg/ha and VectoLex WDG at the 16.79 kg/ha rate, whereas VectoLex WDG did not differ significantly among the 3 application rates. After 3 days posttreatment, percent control dropped quickly for the low and medium rates. Greater than 82% control was achieved by both formulations at 3 days posttreatment in both the VectoLex large-plot and small-plot tests, suggesting a 3-day maximum effective period for these products under conditions found in Arkansas rice fields. Residual activity > 70% was present in plots containing VectoLex WDG at 11.18 and 16.79 kg/ha.

Anopheles and *Psorophora* test

Both VectoLex formulations at the 11.18 kg/ha rate were highly effective against larvae of *Ps. columbiae,* resulting in 100% control during the 1st 24 h posttreatment, whereas less than 20% control was achieved for larvae of *An. quadrimaculatus* within the same plots (Table 2).

DISCUSSION

The lack of extended high residual activity within larger plots could have been due to settling of toxin within the water column, reduced penetration of the canopy during application, and/or low rates of filtration. Aly et al. (1988) reported that at high larval densities, higher amounts of toxin were required to induce an equal amount of mortality compared to the amount of toxin required to induce mortality at lower larval densities because of low filtration rates of suspended bacterial toxins by *An. quadrimaculatus.* In contrast, the VectoBac small-plot tests resulted in 100% mortality for all 3 treatments, which was probably due to consistency of application by hand.

Removing the dead larvae from the sentinel cages may have influenced extended residual activity in our tests involving *B. sphaericus* formulations. Becker et al. (1995) performed a series of laboratory tests that proved that *B. sphaericus* recycles in intact cadavers of *Culex pipiens* L., subsequently increasing spore density. They also pointed out that cadavers must be intact for a period of time and not crushed in order to promote recycling. In order to recycle, the rate at which *B. sphaericus* was applied was more important than the density of live larvae present, which resulted in residual activity detected to 26 days with 100% control in containers with undamaged cadavers.

Although extended residual activity sounds inviting, care must be taken to avoid the buildup of resistance within a mosquito population. Resistance to bacterial compounds has occurred in some mosquito species, but not in *Anopheles* (Jittawadee and Mulla 1996, Nielsen-Leroux et al. 1997). With VectoLex providing 100% control against *Psorophora* and only 20% control against *Anopheles,* a species susceptibility undoubtedly existed in our tests, which also suggested that the compound was still present and active towards 1 species.

Based on our tests, experimental floating formulations of *B. thuringiensis* var. *israelensis* were relatively quick acting at the 5.58 and 11.18 kg/ha rates and well suited for the precise removal of 3rd- to 4th-stage *Anopheles* larvae from an area within 24–48 h, whereas WDG formulations containing *B. sphaericus* required 48–72 h to yield >75% mortality in large-plot tests at an ITU equivalent rate of 11.18 kg/ha. These results were comparable to those of preliminary small-scale tests conducted earlier in the season.

Detecting and targeting the smaller developmental stages (1st- and 2nd-stage larvae) could increase the effectiveness of the tested compounds against *An. quadrimaculatus* in Arkansas and other rice-growing regions, but 2 considerations have the highest priority regarding control of *Anopheles* larvae using biologically derived compounds, that is, a compound must provide a quick kill before settling in the water column or it must remain on or near the water surface for an extended period of time.

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