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EFFICACY OF VECTOLEX® WDG AGAINST *ANOPHELES QUADRIMACULATUS* AND *PSOROPHORA COLUMBIAE* LARVAE IN ARKANSAS AND MISSISSIPPI RICE[1,2]

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ABSTRACT. In 1999, an aerial application of VectoLex® WDG (water-dispersible granules) at 1.68 and 0.56 kg/ha, applied against sentinel 3rd-stage larvae of *Psorophora columbiae* installed in 0.42-ha rice plots 48 h after treatment, provided no control at 72 and 96 h after treatment. Less than 10% reduction was obtained at both rates 8 and 9 days after treatment against larvae of *Ps. columbiae* installed at 7 days after treatment. In a later test, VectoLex WDG manually applied at 5.04 and 1.68 kg/ha to small rice plots containing sentinel 3rd-stage larvae of *Ps. columbiae* and *Anopheles quadrimaculatus* obtained 90 and 97% control of *Ps. columbiae* at both rates, respectively, 24 h after treatment. A 2nd installation of *Ps. columbiae* at 24 h after treatment resulted in 7% and no control at both rates, respectively, even in the presence of larval carcasses from the 1st installation. VectoLex WDG was not effective against *Ps. columbiae* after 24 h after treatment at either rate. Poor control was obtained at both rates against *An. quadrimaculatus* 24 h and 48 h after treatment for both installations. Two types of commercial rice fields containing native populations of larvae of *An. quadrimaculatus* were used for field tests in Cleveland, MS. In 1999, VectoLex WDG, aerially applied at 1.68 and 0.56 kg/ha to 0.2-ha plots in a contoured rice field, produced 81 and 85% reductions in early (neonate and 1st- and 2nd-stage) larvae and 94 and 76% reductions in late (3rd- and 4th-stage) larvae 2 days after treatment, respectively. At 2 days after treatment, means for all 4 developmental groupings (early larvae, late larvae, pupae, and combined stages) were significantly higher in untreated plots. Both VectoLex WDG rates did not differ significantly from one another. At 8 days after treatment, untreated plots contained significantly greater mean numbers of early larvae, late larvae, and combined stages, whereas both VectoLex WDG treatments were not significantly different. In 2000, VectoLex WDG applied at 1.68 kg/ha to two 0.40-ha plots in a precision-leveled field yielded 59 and 100% reductions of early and late larvae, respectively, 2 days after treatment. Reduction of late larvae remained 100% at 8 days after treatment. The numbers of late larvae, pupae, and combined stages were significantly greater in the untreated plot 2 days after treatment. At 8 days after treatment, numbers of early larvae and combined stages were significantly higher in the VectoLex WDG plot, whereas numbers of late larvae were significantly higher in the untreated plot. The differences in susceptibility of *Ps. columbiae* and *An. quadrimaculatus* to VectoLex WDG could be attributed to species differences in larval feeding behavior, body positioning in the water column, and developmental time. In tests in Arkansas, *Ps. columbiae* were controlled more quickly, usually within 24 h of exposure, whereas the percent reduction for *An. quadrimaculatus* in both tests in Cleveland, MS, suggests that control of this species within the region tested required from 48 h up to 8 days of exposure.

KEY WORDS *Anopheles quadrimaculatus, Psorophora columbiae, Bacillus sphaericus,* VectoLex®, water-dispersible granules, larvicide

INTRODUCTION

A series of tests was performed in 1998 to determine the effectiveness of standard and experimental formulations of VectoBac® (Abbott Laboratories, N. Chicago, IL) and VectoLex® (Abbott Laboratories) against larvae of *Anopheles quadrimaculatus* Say confined to sentinel cages in rice fields in Arkansas. During these tests, 48–72 h after treatment was required to obtain >75% mortality of this species at an International Toxic Unit (ITU) equivalent rate of 11.18 kg/ha for VectoLex WDG, a water-dispersible granular formulation containing *Bacillus sphaericus* Neide (serotype H5a5b, strain 2362, 600 *Bs* ITU/mg). At the same rate, VectoLex WDG was found to be highly effective against larvae of *Psorophora columbiae* (Dyar and Knab), resulting in 100% control at 24 h after treatment (Dennett and Meisch 2000).

Lacey et al. (1986) applied a flowable concentrate of *B. sphaericus* (isolate 2362) aerially at rates of 0.58 and 1.17 liter/ha to 0.81-ha (2-acre) rice plots in Arkansas and obtained 71 and 82% reduction, respectively, of larvae of *An. quadrimaculatus* at 48 h after treatment, but at 1 wk after treatment, the corresponding percent reduction dropped to 63 and 40%, respectively.

Recent research conducted by Su and Mulla (1999) showed that a *B. sphaericus* WDG formulation (ABG-6491) at potencies of 350 *Bs* ITU/mg and 630 *Bs* ITU/mg provided 14–20 d of control

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

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of *Culex* in 1.0 × 1.0 × 0.4-m fiberglass tubs with dosages of 0.05–0.10 lb/acre (0.05–0.11 kg/ha). Their dosages were well below those tested by Dennett and Meisch (2000), which suggested that differences exist in susceptibility to Vectolex WDG among mosquito species.

Based on the information we obtained in previous tests in Arkansas, field tests were designed to determine the effects of VectoLex WDG (600 *Bs* ITU/mg) at 2 rates of application on *Ps. columbiae* and on native *An. quadrimaculatus* in 2 types of commercial rice fields in Cleveland, MS, at various times after treatment.

MATERIALS AND METHODS

1999 Psorophora *field test, Stuttgart, Arkansas:* Because larvicidal activity at 24 h after treatment was high in previous tests (Dennett and Meisch 2000), a test was designed to determine efficacy of VectoLex WDG beyond 48 h after treatment in large undisturbed rice plots. VectoLex WDG was applied aerially to 20- to 25-cm-high 'Bengal' rice in 0.42-ha irrigated plots at rates of 0.56 and 1.68 kg/ha at the University of Arkansas Rice Research and Extension Center (RREC), Stuttgart, AR, in June 1999. Both treatments and a control were replicated twice, with a 50-m buffer between treatment plots. Although plots were bordered by contour levees, water flow was taken into consideration before application to prevent the possibility of cross-contamination, resulting in untreated control plots being positioned nearest the effluent. Standing water depth in plots was maintained at 10–15 cm throughout the testing period. Application was made with a Thrush Turbo® airplane (Ayres Corporation, Albany, GA) flying at 177 km/h and 3 m above the rice plots. Wind speed during the applications registered less than 8 km/h. Two floating sentinel cages (Sandoski et al. 1986) were placed in each plot, positioned approximately 30 m from either end. At 48 h after treatment, 10 3rd-stage larvae of *Ps. columbiae* were added to the cages and subsequently covered with tulle cloth secured by rubber bands. Larval mortality was recorded at 3 and 4 days after treatment. At 4 days after treatment, a 2nd installation of fresh 3rd-stage larvae of *Ps. columbiae* was added to the cages, and mortality was recorded at 8 and 9 days after treatment. Data were corrected for control mortality with Abbott's formula and were arcsine transformed before conducting a 1-way analysis of variance (ANOVA; Abbott 1925, Sall and Lehman 1996). Comparisons of means were made with Student's *t*-tests.

1999 Psorophora *and* Anopheles *small-plot test, Stuttgart, AR:* Because of the lack of control against *Ps. columbiae* in the aerial field test, a test was conducted to ascertain efficacy of VectoLex WDG before 48 h after treatment with larvae of both *An. quadrimaculatus* and *Ps. columbiae* in small rice plots treated at rates of 1.68 and 5.04 kg/ha. The test consisted of 9 small rice plots each measuring 58 m^2, each planted in 'Bengal' rice approximately 30–45 cm high and flooded to a water depth of 10–15 cm. Three replicates were performed of both VectoLex WDG rates and untreated controls. Two empty sentinel cages were placed in each plot before applications were made with a CO_2 pressurized 2-liter plastic bottle containing VectoLex WDG mixed in water. Afterwards, ten 3rd-stage larvae of *Ps. columbiae* and *An. quadrimaculatus* were placed in separate cages within each plot. Larvae of *Ps. columbiae* were collected from flooded swales, whereas larvae of *An. quadrimaculatus* were obtained from laboratory cultures maintained at the RREC. Sentinel cages were read at 1 day after treatment, were restocked with fresh larvae, and were read again at 2 days after treatment. Larvae in the 2nd installation were left exposed to intact larval carcasses from the 1st installation. Statistical analysis consisted of correcting for natural mortality and arcsine transformation, followed by a 1-way ANOVA. Student's *t*-tests were used for comparisons of means.

1999 Anopheles *field test, Cleveland, MS.* A commercial rice field containing native populations of larvae of *An. quadrimaculatus* was used for a field test in Cleveland, MS, during June and July 1999. The field contained contour levees and was well flooded to an approximate depth of 10 cm on the day of treatment.

Six plots measuring 137 × 15 m with an area of 0.2 ha were established in the southwestern edge of the field, taking advantage of water flow and levee construction to prevent cross-contamination. Plots were separated from one another by a 15-m buffer strip. Treatments included 2 replicates of untreated controls, and VectoLex WDG aerially applied at 0.56 and 1.68 kg/ha rates.

Untreated control plots were located near the field margin and effluent and were flooded 1st, with the low-rate plots following next, and the high-rate plots located still farther out in the field, away from the direction of water flow. Water levels were consistent throughout most of the test, but began to fluctuate toward the end of the test, with dry areas emerging in portions of the field on July 22.

Aerial application was performed by an Air-Tractor® model 301 (Air Tractor, Olney, TX) equipped with a spray boom fitted with CP® nozzles (CP Products, Tempe, AZ). Aircraft speed was approximately 193 km/h at an altitude of 4.5 m. Final mixture rates were the same as with the Thrush Turbo in the Stuttgart rice test. Pretest dipper counts of larvae of *An. quadrimaculatus* were made before application on June 29 and posttreatment dipper counts were made 2, 8, 14, and 23 days after treatment.

Eighty dips were made for each treatment plot (40 dips/sampler/plot). Dips were made along a central transect with samplers separated by approximately 10 m and a dip being made approximately every 2 m. The average number of larvae collected in untreated control (UTC) plots on the previous sample date over the average number of larvae collected in UTC plots on the next sample date were annotated to provide a general view of larval population change from 1 sample date to the next in areas not impacted by an application. These averages were based on nontransformed totals for a specific larval size class divided by the number of dips made in each treatment on a particular sample date.

For recording purposes, we chose to separate recently hatched 1st-stage larvae (neonates) from older, larger 1st-stage larvae. Developmental stages of *An. quadrimaculatus* collected were divided into 4 groups consisting of early (neonate and 1st- and 2nd-stage larvae), late (3rd- and 4th-stage larvae), pupae, and combined (neonate through pupae). Field data were subjected to a square root transformation, $\sqrt{(y + 0.5)}$, before conducting an ANOVA on developmental groups collected within all treatments on a specific date (Steel and Torrie 1980). Comparisons of means were performed with Tukey–Kramer honestly significant difference (HSD) tests (Sall and Lehman 1996). Additionally, the percent reduction for developmental groups was calculated with a modification of the formula developed by Mulla et al. (1971). Because of periodic rapid shifts in the population of immature *An. quadrimaculatus* and the length of time between sampling dates, dipper counts from preceding sampling dates were used in computations to determine developmental population changes from 1 sampling date to the next, instead of strictly relying on initial pretreatment counts for comparison throughout the entire test. Our justification for this was based on the fact that preceding counts would more accurately reflect larval population changes over the 24-day testing period. The same basic assumptions found in the original formula were also applied to the modified expression. The modified expression is:

$$\text{percent reduction} = 100 - [(UTC_{La} \times TRT_{Nx})/(TRT_{La} \times UTC_{Nx})] \times 100,$$

where UTC_{La} is the mean number of larvae dipped in untreated control plots on the last sampling date, TRT_{La} is the mean number of larvae dipped in treated plots on the last sampling date, UTC_{Nx} is the mean number of larvae dipped in untreated control plots on the next sampling date, and TRT_{Nx} is the mean number of larvae dipped in treated plots on the next sampling date.

2000 Anopheles *field test, Cleveland, MS:* A similar unreplicated field test was conducted in Cleveland, MS, during June and July 2000. A 4.04-ha precision-leveled commercial rice field, flooded to an approximate depth of 10 cm, was chosen for the test. Two plots, measuring 0.40 ha each (64 × 64 m), were established along the central axis of the field, which traveled west to east. The untreated control plot, which began 7.6 m from the western edge of the field, was positioned nearest the effluent to prevent cross-contamination due to water flow. A 35-m buffer strip separated the untreated control plot and the WDG plot positioned farther east along the axis.

VectoLex WDG was applied at 1.68 kg/ha with methods previously described for the 1999 Cleveland test. Pretreatment dipper counts were made before application on June 27, and posttreatment counts were made 2, 8, 14, and 23 days after treatment.

The sampling scheme remained identical to that of the 1999 Cleveland field test, with the exception that only 1 large plot was sampled for each treatment, resulting in 160 dips (40 dips/person/transect × 2 persons × 2 transects) per treatment per sampling day. Field data were subjected to identical handling and analyses as previously outlined in the 1999 test in Cleveland, MS.

RESULTS

In 1999, the mean number of live larval *Ps. columbiae* in large foundation seed rice plots at the RREC remained consistently high throughout the test. We observed 100% survival of larval *Ps. columbiae* at rates of 0.56 and 1.68 kg/ha VectoLex WDG, respectively, at 72 and 96 h after treatment. No significant differences were detected in the 2nd installation, with less than 10% reduction at both rates at 8 and 9 days after treatment. Because of the possibility that larvae sampled 72 and 96 h after the 1st installation of larvae at 48 h after treatment were exposed to little if any active ingredient, a small plot test was instigated in an attempt to ascertain efficacy after 48 h aftertreatment.

Mortality of *Ps. columbiae* was high at both rates in the 1999 RREC small plot test 24 h after treatment, but, interestingly, the addition of fresh *Ps. columbiae* to cages with intact carcasses resulted in very low mortality. In the 1st installation, 90% control was obtained with the high rate of 5.04 kg/ha, whereas 97% control was observed at the lower rate of 1.68 kg/ha at 24 h after treatment. No significant differences ($P > 0.05$) were detected between treatments. Significant differences were not found in the 2nd installation of *Ps. columbiae* that resulted in 7% control at the high rate, and no control at the low rate, even in the presence of intact larval carcasses.

Although excellent control was obtained against larval *Ps. columbiae* 24 h after treatment with both rates in small plot tests, *An. quadrimaculatus* survived well at both rates, with no significant differences in the means of both treatments for both installations. No control (0%) was obtained at either rate for *An. quadrimaculatus* at 24 or 48 h after treatment in either installation.

VectoLex WDG was not effective against *Ps. columbiae* after 24 h at either rate. Mortality of *An.*

Table 1. Percent reduction of early- and late-stage larvae of *Anopheles quadrimaculatus* at 2, 8, 14, and 23 days after treatment with VectoLex® WDG (1.68 and 0.56 kg/ha) in a rice field with contour levees during June and July 1999 in Cleveland, MS.

Larval stage[1]	Rate (kg/ha)	Average no. larvae/dip[2]	Percent reduction[3]	Days after treatment
E	1.68	1.15/2.06	81	2
E	0.56	1.15/2.06	85	2
L	1.68	1.02/0.90	94	2
L	0.56	1.02/0.90	76	2
E	1.68	2.06/0.50	0	8
E	0.56	2.06/0.50	0	8
L	1.68	0.90/0.64	0	8
L	0.56	0.90/0.64	0	8
E	1.68	0.50/0.75	0	14
E	0.56	0.50/0.75	0	14
L	1.68	0.64/0.18	0	14
L	0.56	0.64/0.18	0	14
E	1.68	0.75/0.98	12	23
E	0.56	0.75/0.98	0	23
L	1.68	0.18/0.41	9	23
L	0.56	0.18/0.41	0	23

[1] Larval classification, placing neonate, 1st, and 2nd instars into early (E), and 3rd and 4th instars into late (L).

[2] For a given larval class in a specific row, the average number collected in untreated control plots (UTC) on the previous sample date over the average number collected in UTC plots on the next sample date. Averages were based on nontransformed totals for a larval class divided by the number of dips made in each treatment on each sample date ($n = 160$).

[3] Based on modifications of the formula developed by Mulla et al. (1971).

quadrimaculatus was moderate. The numbers of larval *An. quadrimaculatus* that survived indicates that larvae did not ingest enough active ingredient to induce mortality at the rates tested.

In the 1999 Cleveland test, 81 and 85% reductions in early larvae and 94 and 76% reductions in late larvae were obtained 2 days after treatment at 1.68 and 0.56 kg/ha, respectively (Table 1). Little if any control was obtained at later dates (Table 1). Two days after treatment, the numbers of all 4 developmental groupings were significantly greater in untreated plots than in treated plots. The control did not differ significantly between both VectoLex WDG rates (Table 3). At 8 days after treatment, with the exception of pupae, which did not differ statistically among treatments, untreated plots contained significantly higher mean numbers of early and late larvae and combined stages. Mortality from both VectoLex WDG treatments did not differ significantly ($P > 0.05$) during this period (Table 3). Fourteen days after treatment, higher mean counts of early larvae were detected in plots treated at higher rates, whereas no significant differences were present in mean counts of late larvae in all treatments during the same interval ($P > 0.05$). Additionally, the mean number of pupae was significantly higher in lower-rate plots compared to plots containing higher VectoLex WDG rates, but did not differ from untreated plots (Table 3). Although no significant differences were noted in either pupae

Table 2. Percent reduction of early- and late-stage larvae of *Anopheles quadrimaculatus* larvae at 2, 8, 14, and 23 days after treatment with VectoLex® WDG (1.68 kg/ha) in a precision-leveled rice field during June and July 2000 in Cleveland, MS.

Larval stage[1]	Rate (kg/ha)	Average no. larvae/dip[2]	Percent reduction[3]	Days after treatment
E	1.68	0.42/0.52	59	2
L	1.68	0.22/0.39	100	2
E	1.68	0.52/0.41	0	8
L	1.68	0.39/0.06	100	8
E	1.68	0.41/1.15	84	14
L	1.68	0.06/0.93	0	14
E	1.68	1.15/0.63	0	23
L	1.68	0.93/0.28	58	23

[1] Larval classification, placing neonate, 1st, and 2nd instars into early (E), and 3rd and 4th instars into late (L).

[2] For a given larval class in a specific row, the average number collected in untreated control plots (UTC) on the previous sample date over the average number collected in UTC plots on the next sample date. Averages were based on nontransformed totals for a larval class divided by the number of dips made in each treatment on each sample date ($n = 160$).

[3] Based on modifications of the formula developed by Mulla et al. (1971).

Table 3. Comparisons of early larval, late larval, pupal, and combined developmental stages of *Anopheles quadrimaculatus* collected at 2, 8, 14, and 23 days after treatment in a contoured rice field containing untreated control (UTC) plots and plots treated with VectoLex® WDG at rates of 1.68 (high) and 0.56 (low) kg/ha in Cleveland, MS, during June and July 1999.

Days after treatment	Treatment	Developmental stages collected[1]			
		Early larvae	Late larvae	Pupae	Combined
2	UTC	1.45 A	1.07 A	0.77 A	1.73 A
	High	0.82 B	0.71 B	0.71 B	0.83 B
	Low	0.80 B	0.74 B	0.71 B	0.84 B
Standard error		0.034	0.023	0.008	0.039
8	UTC	0.94 A	0.97 A	0.73 A	1.18 A
	High	0.84 B	0.83 B	0.72 A	0.97 B
	Low	0.84 B	0.82 B	0.72 A	0.97 B
Standard error		0.023	0.027	0.008	0.034
14	UTC	1.02 B	0.79 A	0.72 AB	1.11 B
	High	1.22 A	0.78 A	0.70 B	1.27 A
	Low	0.94 B	0.77 A	0.73 A	1.03 B
Standard error		0.037	0.016	0.006	0.039
23	UTC	1.10 B	0.89 AB	0.72 A	1.25 A
	High	1.30 A	0.86 B	0.72 A	1.41 A
	Low	1.15 B	0.98 A	0.73 A	1.37 A
Standard error		0.042	0.027	0.008	0.048

[1] Treatment means and associated standard errors based on an analysis of variance of transformed data (n = 160). For a given day after treatment, means within columns for a specific developmental classification (early larvae, late larvae, pupae, combined) followed by the same uppercase letter(s) are not significantly different from one another ($P > 0.05$).

or combined stages 23 days after treatment ($P > 0.05$), significantly greater numbers of late larvae were found in lower-rate plots compared to higher-rate plots, but not in untreated plots. Early larvae were significantly greater in plots treated at higher VectoLex WDG rates during the same interval (Table 3).

During the 2000 Cleveland test, 59 and 100% reductions of early and late larvae were observed 2 days after treatment at 1.68 kg/ha. Although reduction of late larvae remained 100% at 8 days after treatment, no reductions of early larvae occurred. An 84% reduction of early larvae was witnessed, with no reductions in the number of late larvae 14 days after treatment. At 23 days after treatment, a 58% reduction of late larvae was observed (Table 2).

The mean counts of late larvae, pupae, and combined stages were significantly greater in the untreated plot, whereas no differences ($P > 0.05$) were detected in the number of early larvae in either treatment 2 days after treatment (Table 4). Al-

Table 4. Comparisons of early larval, late larval, pupal, and combined developmental stages of *Anopheles quadrimaculatus* collected at 2, 8, 14, and 23 days after treatment in a precision-leveled rice field containing untreated control (UTC) plots and plots treated with VectoLex® WDG at a rate of 1.68 (high) kg/ha in Cleveland, MS, during June and July 2000.

Days after treatment	Treatment	Developmental stages collected[1]			
		Early larvae	Late larvae	Pupae	Combined
2	UTC	0.94 A	0.88 A	0.76 A	1.51 A
	High	1.01 A	0.71 B	0.70 B	1.01 B
Standard error		0.029	0.018	0.009	0.032
8	UTC	0.89 B	0.73 A	0.71 A	0.91 B
	High	1.26 A	0.70 B	0.70 A	1.26 A
Standard error		0.051	0.008	0.002	0.052
14	UTC	1.18 A	1.08 B	0.71 A	1.49 A
	High	1.05 B	1.20 A	0.71 A	1.49 A
Standard error		0.038	0.041	0.005	0.048
23	UTC	0.98 B	0.81 A	—	1.06 B
	High	1.18 A	0.78 A	—	1.24 A
Standard error		0.037	0.023	—	0.044

[1] Treatment means and associated standard errors based on an analysis of variance of transformed data (n = 160). For a given day after treatment, means within columns for a specific developmental classification (early larvae, late larvae, pupae, combined) followed by the same uppercase letter(s) are not significantly different from one another ($P > 0.05$).

though the mean numbers of pupae in both plots 8 days after treatment were not statistically different from each other, early larvae and combined stages were significantly greater in the VectoLex WDG plot. Numbers of late larvae were significantly greater in the untreated plot (Table 4). At 14 days after treatment, no differences were detected in the means of pupae or combined stages in either treatment ($P > 0.05$). However, a significantly greater number of late larvae was found in the VectoLex WDG plot, whereas early larvae were significantly greater in the untreated plot (Table 4). No pupae were collected 23 days after treatment, and no significant difference was found between the means of late larvae in either treatment, although statistically greater means of early larvae and combined stages were found in the VectoLex WDG plot (Table 4).

DISCUSSION

Clearly, a distinct difference occurred in the susceptibility of *Ps. columbiae* and *An. quadrimaculatus* to VectoLex WDG. This may have been due, in part, to species differences in larval feeding behavior, body positioning within the water column, and developmental time. *Psorophora columbiae* were more quickly controlled, usually within 24 h, but residual activity due to bacterial recycling was not observed in this species. Reductions at 8 days after treatment may or may not have been attributed to residual activity of VectoLex WDG.

Mortality in both Cleveland tests suggested that control of *An. quadrimaculatus* within the region tested was probably limited to 48 h after treatment. The 100 and 59% reductions of late larvae seen within the Cleveland 2000 test at 8 and 23 days after treatment, respectively, most probably were attributed to decreases in the dipper counts from untreated plots (Table 2). Numbers of late larvae in both tests were significantly fewer in VectoLex WDG plots at 8 days after treatment, suggesting that either some residual activity or a slow replacement rate of *An. quadrimaculatus* may have been occurring.

Within certain mosquito species, *B. sphaericus* uses intact larval carcasses for growth and disperses into the water column upon carcass disintegration, whereas larval carcasses may also provide some protection from degradation induced by sunlight (Des Rochers and Garcia 1984). Direct sunlight may not be the prime consideration in terms of degradation of the bacteria or its toxic moiety, provided that an aerial application of *B. sphaericus* penetrates a dense rice canopy and strikes the water. However, water temperature may play an important role. In rice in Arkansas and Mississippi during the summer, larval carcasses of both species break down quickly at high temperatures, thus preventing bacterial recycling.

Applications of some *B. sphaericus* formulations for control of urban *Culex* populations are made to relatively small areas with high larval densities, both of which are necessary for bacterial recycling and extended residual activity. High larval densities in relatively confined areas are not commonplace with anopheline species in rice fields. In contrast, when confined to relatively small areas, larval *Anopheles punctipennis* (Say) in waste tires were not effected by applications of VectoLex CG corncob granules containing 50 *Bs* ITU/mg (Siegel and Novak 1997). Historically, *An. punctipennis* also were the least affected by *B. sphaericus* of Arkansas species (Groves and Meisch 1996). Previous findings combined with recent research conducted in both Arkansas and Mississippi suggest that *Anopheles* spp. common to both areas may not be effectively controlled over extended periods when using current *B. sphaericus* formulations.

One possible reason that *B. sphaericus* is less effective on riceland *Anopheles* spp. is that *An. quadrimaculatus* larvae do not occur in either relatively confined areas or at high densities compared with urban *Culex* spp. found in septic ditches or catch basins. In a study performed in Zaire, Karch et al. (1992) determined that the number of *B. sphaericus* spores present in rice fields over 10 treatment cycles decreased more than 50 and 70% at 7 and 14 days after treatment, respectively, when using a granular VectoLex (ABG-6185) formulation. Furthermore, *Anopheles* spp. larvae feed at the thin air–water interface at rates up to 20 times less than *Aedes* or *Culex* (Aly et al. 1987, 1988; Merritt et al. 1992; Walker and Merritt 1993; Wallace and Merritt 1980).

In summary, Vectolex WDG provided adequate control of 2nd-, 3rd-, and 4th-stage larvae of *An. quadrimaculatus* 48–72 h after treatment, but provided little, if any, control 8 days after treatment. Neonates and pupae are life stages that are difficult to assess with regard to efficacy. Neonates may have hatched only recently and thus had not yet been affected. Pupae are extremely difficult to monitor accurately by dipping, but were included, because numbers collected may have reflected 3rd to 4th instars of *An. quadrimaculatus* that could have ingested a sublethal dose. We have not yet found evidence of bacterial recycling in Arkansas and Mississippi rice fields with *An. quadrimaculatus* that would indicate major extended residual activity beyond 48–72 h after treatment with the application rates and methodologies used.

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