ASSESSMENT OF INDUSTRIAL FORMULATIONS OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS*[1, 2]

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ABSTRACT. Laboratory and field comparisons were made with 5 formulations of *Bacillus thuringiensis* var. *israelensis* de Barjac *(Bti)*. Against second-stage larvae of *Aedes aegypti* the activity (International Toxic Units per mg) of the test materials was: Abbott Laboratories' ABG 6108 WP (6406-125), 651 ITU, ABG 6108 WP (6478-199), 567 ITU, and ABG 6109 (6406-122), 286 ITU; Biochem Products' 666 PM 50, 3482 ITU; and Sandoz Corporation's SAN 402 I WDC, 688 ITU. Small plot field studies revealed that *Bti* at 0.7-5.2 kg/ha provided complete control of *Ae. aegypti* depending upon formulation. Application of 1, 1.5, and 4 kg/ha of SAN 402 I WDC, Biochem 666 PM 50, and ABG 6108 WP (6478-199), respectively, provided complete control of caged larvae of *Psorphora columbiae* in rice field studies in Arkansas; higher levels were required for *Anopheles quadrimaculatus*. ABG 6108 WP (6406-125) gave 99% and 100% control of *Aedes taeniorhynchus* in Florida at 2.5 and 5 kg/ha, respectively. In some cases the relative activity of the *Bti* formulations was reversed or the differences were narrowed under field conditions compared to the laboratory results.

INTRODUCTION

The bacterium *Bacillus thuringiensis* var. *israelensis* de Barjac *(Bti)* has been shown to be effective against mosquito larvae (Goldberg and Margalit 1977, de Barjac and Coz 1979, Garcia and DesRoches 1979). Due to the promising results with this bacterium, several commercial organizations have developed experimental formulations for use against mosquito larvae. This paper reports the results of laboratory and field tests conducted with 5 industrially prepared formulations of *Bti*.

MATERIALS AND METHODS

SPECIFIC ACTIVITY OF EXPERIMENTAL FORMULATIONS. Assays were conducted under laboratory conditions to determine the activity of the following experimental formulations: SAN 402 I WDC, a semiliquid formulation from Sandoz, Inc.; ABG 6108 WP (lot 6406-125), ABG 6108 WP (lot 6478-199), and ABG 6109 WP (lot 6406-122), wettable powder formulations from Abbott Laboratories; and 666 PM 50, a wettable powder from Biochem Products. In addition to these compounds IPS-78, a tentative international standard prepared and distributed by the Pasteur Institute (de Barjac and Larget 1979), was also evaluated.

The primary assays were conducted with second stage larvae (L2) from the laboratory strain of *Aedes aegypti* (Linnaeus) maintained at Gainesville. Twenty 24- to 27 hr-old larvae were placed in a

[1] This research was conducted as part of the Cooperative Regional Research Project on the Biology and Control of Riceland Mosquitoes, S-122.

[2] Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

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100 ml waxed paper cup in a small volume of well water containing 1 ml of a 1% suspension of hog liver and brewer's yeast (3:2). A 10 ml aliquot of the appropriate stock suspension of the test formulation was then pipetted into the cup. Enough water was then added to bring the total volume up to 100 ml. In the initial test with each material 7 to 9 concentrations were run in triplicate (60 larvae) and the results were used to select a range of 9 concentrations for subsequent testing. Nine concentrations were used to assure an adequate distribution of data points above and below 50%, to satisfy the statistical analysis. The results obtained from 3 replicates, each containing triplicate cups, were converted to log probits and the LC-50 values estimated by probit analysis.

The specific activity of each material was determined by comparing its LC-50 value for a 24 hr exposure with that of the IPS-78 standard. Using 1,000 International Toxic Units (ITU) per mg as an arbitrary value for the activity of IPS-78, the activity (ITU mg) was calculated as follows:

$$\frac{\text{IPS-78} \quad \text{LC-50}}{\text{Test compound LC-50}} \times 1000$$

A similar series of tests was conducted with IPS-78 using late third-early fourth stage larvae (L3/4) of *Ae. aegypti* and *Anopheles quadrimaculatus* Say.

SMALL PLOT OUTDOOR ASSAYS. Tests with the experimental formulations were conducted in the summers of 1979 and 1980. Artificial ponds lined with St. Augustine sod over 8 mil polyethylene sheeting were used. The ponds were 80 cm deep and, when filled to a depth of 15 cm, the surface area ranged from 3.7 to 5.6 m^2 (mean: 3.8 m^2). They were flooded the day before treatment and the experimental materials applied by pouring an aqueous suspension of the formulation into the pond and then stirring to mix.

Water samples were collected before treatment, immediately after treatment, 6 and 24 hr after treatment, and at 24 hr intervals thereafter. The samples were transported to the laboratory where foreign matter was removed by filtering it through a coarse screen (7 strands per cm). Each sample was then divided into four 100-ml aliquots for tests against 2 sets of 20 each of *Ae. aegypti* L3/4 and *An. quadrimaculatus* L3/4. Living larvae were counted after 24 and 48 hr of exposure. Twenty additional larvae were introduced into test aliquots that had no survivors after 24 hr. Each dose tested was replicated, and separate, untreated control plots were assayed with each replicate. At the completion of each test the water was pumped out of the ponds and the ponds were maintained dry until needed for subsequent tests.

RICE FIELD ASSAYS. Additional tests were conducted in the summers of 1979 and 1980 in experimental rice plantings at the Arkansas Rice Experiment Station in Stuttgart. The plots measured 6.1 m square, were bordered with a strip of rice, and included an 80 to 90 cm unplanted area surrounding a central 3.6 m square planting of rice in the center. At the time of these tests the rice was ca. 45 cm high. The plots were bordered by earthen levees and were interconnected by pipes running through the levees; these pipes were sealed prior to treatment to prevent the flow of water between plots.

Applications of *Bti* were made using compressed-air Hudson X-pert® sprayers equipped with pressure gauges and Hudson 153-400 flat fan spray nozzles. Aqueous suspensions of the formulations were prepared in the field just prior to application. A concentrated suspension of preweighed test material was mixed in a small volume of well water to make a slurry, which was added to sufficient well water in the spray tank to make a total of 4 liters. To assure uniform coverage of the plot, the application rate was calibrated to deliver 1 liter in each of 4 swaths, which ran 1.5 m inside and parallel to each levee. Thus, the entire plot was sprayed twice with an overall application of 4 liters of aqueous suspension of the test material.

Larvae of *Psorophora columbiae* Dyar and Knab were obtained by flooding predetermined oviposition sites prior to testing. When the larvae reached the third instar they were brought to the laboratory. *Anopheles quadrimaculatus* larvae were collected from nearby rice fields. Both species were maintained in enamel pans and glass trays using the original collection water. At treatment time they were transferred to paper cups, given a small amount of Tetramin® fish food, and placed in PVC cylinders in the plots. The cylinders were ca. 15-22 cm long and 10 cm in diameter and were capped on the bottom with a 35 mesh nylon screen. After the plot was treated, the larvae were introduced into the PVC cylinders and these were then covered with cheesecloth. When immersed into the plot the water filled the cylinder to a depth of 6 to 10 cm. The cylinders had six 1 cm holes, screened with nylon mesh, near the bottom of the cylinder to allow water movement. Ten *Ps. columbiae* L3 were placed in one cylinder at the center of the plot and in 1 or 2 similar containers at the edge of the central rice planting. In some trials an additional cylinder containing 5 *An. quadrimaculatus* (L4) and 5 *Ps. columbiae* (L4) was placed at the edge of the plot. At 24 hr posttreatment fresh cylinders containing 5 or 10 *Ps. columbiae* (L3) were placed at the edge of the plot to assess residual activity of the treatments.

SALT MARSH MOSQUITO ASSAYS. Unusually dry conditions prevented salt marsh mosquito assays in 1980, but in 1979 we were able to test one *Bti* formulation in flooded swales in a citrus grove at Vero Beach, Florida. The flooded areas in the swales measured 1.5 to 3.0 m wide and up to 30 m in length and contained an average of 4 to 45 *Ae. taeniorhynchus* (Wiedemann) (L3) per dip at the time of treatment. Pretreatment larval density was determined by counting the larvae in 17 to 30 dips; posttreatment counts were made ca. 18 hr later using 20 dips per plot. The swales were treated with the equipment described in the previous section by uniformly spraying 165 ml per linear meter of swale.

RESULTS AND DISCUSSION

A wide range in the activity of the experimental compounds was detected in the laboratory assays (Table 1). Biochem 666 PM 50 was the most potent with an activity of 3482 ITU/mg. The other formulations were less active than the standard. Probit analyses revealed the following requirements (mg/liter) to obtain 90% control of *Ae. aegypti* (L2): ABG 6108 WP (6406-125), 0.460; ABG 6108 WP

Table 1. Activity of *Bacillus thuringiensis* var. *israelensis* formulations.

Material	LC-50 (mg/liter) *Aedes aegypti* L2	*Aedes aegypti* L3/4[a]	*Anopheles quadrimaculatus* L3/4[a]	Activity (ITU/mg[b])
ABG 6108 WP (6406–125)	0.128	0.392	1.997	651
ABG 6108 WP (6478–199)	.125	.450	2.293	567
ABG 6109 WP (6406–122)	.291	.892	4.545	286
Biochem 666 PM 50	.022	.073	0.373	3482
SAN 402 I WDC	.121	.371	1.890	688
IPS 78 (Standard)	.083	.255	1.300	1000

[a] LC–50 for formulations estimated by comparison of activity determined experimentally with IPS–78.

[b] Activity for formulations determined by comparison of observed LC–50 with LC–50 of IPS 78, which has arbitrary value of 1000 International Toxic Units per mg (ITU/mg).

(6478-199), 0.406; ABG 6109 WP (6406-122), 1.09; Biochem 666 PM 50, 0.036; SAN 402 I WDC 0.294; IPS 78, 0.223.

The laboratory assays with IPS-78 revealed differences in susceptibility between larval stages and species. *Ae. aegypti* L3/4 required 3 times as much *Bti* for 50% mortality as did L2. *Anopheles* spp. tend to be less susceptible than other mosquitoes and in these studies the LC-50 for *An. quadrimaculatus* (L3/4) was 5 times greater than that required for *Ae. aegypti* (L3/4).

In the bioassays with aliquots of water from small outdoor plots (Table 2) Biochem 666 PM 50 and SAN 402 I WDC required only 0.7 kg/ha to provide 100% mortality of *Ae. aegypti* L3. In parallel tests ABG 6108 (6478-199) and ABG 6109 (6406-122) gave similar results at 2.8 and 2.6 kg/ha, respectively. However, the relatively lower level of activity of ABG 6109 (6406-122) is revealed in its more rapid degradation; the 6-hr posttreatment collections killed less than either of the other 2 Abbott formulations at most equivalent dosages. ABG 6108 (6406-125), which had an activity (651 ITU/mg) more than twice that of ABG 6109 (6406-122), was somewhat less effective than ABG 6109 (6406-122) at the lowest comparable dosage tested (2.6 kg/ha). However, it persisted better than either of the other 2 Abbott materials at most dosage levels. The trends were similar against *An. quadrimaculatus* L4 but the period of residual

Table 2. Bioassay of small plots treated with *Bacillus thuringiensis* var. *israelensis* formulations (means of 2 replications, each with duplicate samples of 20 larvae/sample; data adjusted by Abbott's formula).

Material	Dosage kg/ha	24-hr Mortality (%) Aliquots collected at indicated hr posttreatment 0	6	24	48	72	96	120
		Aedes aegypti L3						
ABG 6108 WP (6406–125)	2.6	83	3					
	5.2	100	97	95	33			
	10.4	100	95	87	57	97	94	40
	20.8	100	100	99	100	83	59	48
ABG 6108 WP (6478–199)	1.4	97	53	1				
	2.8	100	45	0				
	5.2	100	15					
ABG 6109 WP (6406–122)	1.4	97	3					
	2.6	100	6					
	5.2	99	55	41				
	10.4	100	67	9				
	20.8	100	59	85	53			
Biochem 666 PM 50	0.35	99	6					
	0.7	100	18					
	1.4	100	100	3				
	2.8	100	95	0				
SAN 402 I WDC	0.28	62	3					
	.7	100	8					
	1.4	100	40					
	2.8	100	67	0				
	4.1	100	100	23				
	5.5	100	100	22				
	11.0	100	100	79	0			

Table 2. (Continued).

Material	Dosage kg/ha	24-hr Mortality (%) Aliquots collected at indicated hr posttreatment 0	6	24	48	72	96	120
		Anopheles quadrimaculatus L4						
ABG 6108 WP	2.6	58	0					
(6406–125)	5.2	100	53	51				
	10.4	100	54	39				
	20.8	100	92	89	95[a]		48[a]	
ABG 6108	1.4	59	32					
(6478–199)	2.8	86	6					
	5.2	100	1					
ABG 6109 WP	1.4	54	0					
(6406–122)	2.6	94	4					
	5.2	98	18					
	10.4	100	49					
	20.8	99	50	64	29			
Biochem	0.35	88	0					
666 PM50	0.7	90	0					
	1.4	98	68					
	2.8	100	35					
SAN 402 I WDC	0.28	3						
	.70	88	0					
	1.4	91	21					
	2.8	97	50	3				
	4.1	100	98	0				
	5.5	99	99	0				
	11.0	100	99	44	3			

[a] Average of duplicate samples from one test.

effectiveness was reduced, probably because of the greater tolerance of *An. quadrimaculatus*.

There was a more rapid loss of activity in the ponds than in the laboratory. In those tests in which larvae were replaced in the laboratory bioassay at 24 hr postcollection, mortality was usually higher than that observed from collections taken from the ponds at the same time. For example, samples collected immediately after the ponds were treated with SAN 402 I WDC at 0.7, 2.8, and 4.1 kg/ha gave complete kill of *Ae. aegypti* introduced 24 hr later (24 hr after treatment) whereas samples collected from the ponds 24 hr after treatment gave 0, 0, and 23% kill, respectively. This was probably due to the gradual settling of the spores and crystals to the bottom of the ponds, where they were less available to larvae and sampling. The only short-term exception to the lower activity with time was ABG 6108 (6406-125) applied at 20.8 kg/ha, which gave 99 to 100% kill of *Ae. aegypti* up to 48 hr posttreatment.

In most of the tests, the effectiveness of the treatments that gave 99 to 100% kill in the bioassays of treated pond water was verified in the ponds by visual observation and by dipping for the naturally-occurring species (*Culex salinarius* Coquillett, *Cx. nigripalpus* Theobald, and *Ps. columbiae*). Larvae of these species were killed within 6 to 24 hr; however, larvae that hatched from egg rafts present at the time of treatment frequently survived to pupation, probably because the spores and crystals had settled to the bottom prior to the time they started

feeding. The only naturally-occurring species of mosquito not affected was *Ps. howardii* (Coq.). Larvae of this species were observed 24 and 48 hr posttreatment in ponds that were treated at the highest dosage tested.

There was no indication of a cumulative residual effect in the plots. Mortality in bioassays of water samples collected before treatment from ponds that had been treated previously on various occasions over a 113-day period with a cumulative total of up to 14 gm of *Bti*, was invariably less than that in the controls (<10%).

The tolerance of *An. quadrimaculatus* larvae was again observed in exposures to *Bti* in the rice plantings (Table 3). SAN 402 I WDC at 9.6 kg/ha failed to give complete control of *An. quadrimaculatus* within 48 hr although all the *Ps. columbiae* larvae of the same stage (L4) were killed; ABG 6109 (6406-122) produced a similar response. In the rice plantings SAN 402 I WDC and Biochem 666 PM 50 were about equally effective and provided greater control than the other materials. At the higher doses tested all the materials exhibited some residual activity. Although these tests were done under artificial conditions, i.e., with larvae placed in cylinders, the relative effectiveness of the formulations could be accurately assessed. In practice, one would expect actual dosage requirements to be somewhat lower because free-roaming larvae would encounter and ingest more *Bti* spores and crystals than caged larvae.

Tests with ABG 6108 (6406-125) were not conducted in the rice plantings because studies were being conducted

Table 3. Effect of *Bacillus thuringiensis* var. *israelensis* on larvae of *Psorophora columbiae* and *Anopheles quadrimaculatus* in rice plantings at Stuttgart, Arkansas, (means of 2–3 replicates).

Material	Dosage (kg/ha)	Percentage control							Residual activity
		Psorophora				*Anopheles*			
		L3		L4					% Control of L3 *Psorophora* introduced 24 hr posttreatment
		24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	120 hr	
ABG 6109 WP	10.8	100	100	100	100	20	40	50	60
(6406–122)	21.5	100	100	100	100	40	80	90	55
ABG 6108 WP	1.0	52	63						0
(6478–199)	2.0	70	83						0
	4.0	100	100						10
	8.0	100	100						10
Biochem	1.0	97	97						0
666 PM 50	1.5	100	100						7
	2.0	100	100						10
	4.0[a]	100	100						40
SAN 402 I	0.5	77	82						0
WDC	0.75	87	98						0
	1.0	100	100						0
	1.17	96	100						0
	1.5	100	100						0
	9.6	100	100	100	100	30	90	100	100
	19.1	100	100	100	100	100	100	100	100
Control[b]	0.0	1	6	0	0	0	0	50	0

[a] 1 Replication.
[b] 4–9 Replications.

concurrently with that material by other workers (Hembree et al. 1980). In their tests the bacteria were applied in a manner calculated to produce a uniform concentration (ppm) throughout the planting, which differs in depth from the outer edge to the central area. They obtained 95 and 100% control of L1 and L2 *Ps. columbiae* at treatment levels equivalent to 6.2 and 11.2 kg/ha, respectively. Since the younger larvae are more susceptible, it is reasonable to assume that 6.2 kg/ha would provide less than 95% control of L3 and 11.2 kg/ha might provide complete control. This comparison suggests that ABG 6109 (6406-122) and ABG 6108 (6406-125) might be similar in immediate effect. Both materials provided some mortality in larvae introduced 24 hr posttreatment, indicating residual activity at those treatment levels.

ABG 6108 (6406-125) was effective against *Ae. taeniorhynchus* in flooded swales in the citrus grove in Vero Beach, Florida. Applications were made when the larvae were late in the third instar. When mortality observations were made ca. 18 hr later, some pupation had already occurred in the control plots. At 2.5 kg/ha 99% control was achieved and complete control occurred at 5 kg/ha.

The results presented show that each formulation assessed is active against mosquito larvae within the dose range tested, that there are differences in susceptibility related to larval stage and species, and that at the higher dosages tested residual activity was detected. Finally, the tests revealed that the relative activity of some of the test formulations was dependent on the test situations; under field conditions there were some reversals and narrowing of differences in activity that had been observed under laboratory conditions.

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