LABORATORY TESTS TO EVALUATE THE POTENTIAL EFFICACY OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* FOR USE AGAINST MOSQUITOES[1]

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ABSTRACT. *Bacillus thuringiensis* var. *israelensis (Bti)* was about 85 × more active against 4-day-old larvae of *Aedes aegypti* in distilled water than in pond water (LC-50's of 0.7 vs. 59.7 μg/ml); also, the difference in LT-50 for 1.0 μg/ml was almost 30-fold. An increase in the concentration of pond-water-sediment resulted in a corresponding decrease in activity of *Bti*. About one-half of the *Bti* activity was bound by a sediment concentration of 2%. *Bti* suspended in either pond or distilled water was inactivated after 24 hours' exposure to simulated sunlight-UV. There was no apparent loss in the insecticidal activity of an aqueous suspension of *Bti* held at 30° ± 1°C for more than 100 days. A water salinity of 0.5% had no apparent effect on the larvicidal activity of *Bti*, nor was activity affected in distilled or natural pond water at pH of 4.0, 6.6, and 10.0. No increase in activity of *Bti* due to regrowth after suspension in water was detected. Presence of food or of *Bti*-killed mosquito larvae increased both the rate and extent of mortality. Larval feeding depleted the total activity of a suspension of *Bti*; less than 1% was present after feeding by 3 serial populations of larvae. Suspensions of *Bti* settle in still water; differences in activity between a top and bottom sample could be detected after 1 hr of standing. All available laboratory data indicate that var. *israelensis* is a very promising candidate microbial insecticide for control of mosquitoes.

INTRODUCTION

Many species of entomopathogens have been suggested for development as microbial insecticides against mosquitoes—fungi: *Aspergillus* spp., *Beauveria bassiana, Coelomomyces* spp., *Culicinomyces* spp., *Entomophthora* spp., *Lagenidium giganteum, Metarrhizium anisopliae*; protozoa: *Nosema algerae, Thelohania* spp.; bacteria: *Bacillus sphaericus, Bacillus thuringiensis, Bacillus* spp.; viruses: iridescent virus, nuclear-polyhedrosis virus (Steinhaus and Jenkins 1960, Briggs 1963, 1975; Laird 1971). None has generated more interest than the recently isolated bacterium *Bacillus thuringiensis* var. *israelensis* (Goldberg and Margalit 1977, de Barjac 1978). Variety *israelensis* was more active against species of *Aedes, Anopheles, Culex,* and *Uranotaenia* than any other variety of *B. thuringiensis*, and its rapidity of kill approaches that obtained with some chemical insecticides (Goldberg and Margalit 1977, de Barjac 1978, Garcia and Desrochers 1979, Ignoffo et al. 1980).

There are 3 prerequisites to the development of a candidate microbial insecticide (Ignoffo 1965, 1967): production feasibility, safety to non-target animals and plants, and field efficacy. Production of var. *israelensis* will probably not be a problem; it will probably follow a protocol similar to that used to produce var. *kurstaki* (Ignoffo et al. 1970). In addition, there is no reason to believe that var. *israelensis* is any more hazardous to man and the environment than var. *kurstaki*, which has been used for more than 20 years and has a proven record of safety. The remaining prerequisite to development is efficacy against the target pest. The objective of the present experiments was to evaluate the potential efficacy of var. *israelensis* by defining those environmental factors that may affect its field effectiveness against mosquitoes.

[1] Mention of a proprietary product in this paper does not constitute a recommendation for use by the USDA.

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[5] Mean ± standard error of the mean throughout text and tables.

GENERAL MATERIALS AND METHODS

The experimental preparation of *Bacillus thuringiensis* var. *israelensis (Bti)* serotype H-14, that we used, designated AB-6108 (Lot No. 6406–18), was a spray-dried, on formulated technical powder provided by T. L. Couch (Abbott Laboratories, Inc., North Chicago, Illinois 60064). This preparation had a total spore count and a viable spore count (pour plates incubated for 18 hr at 30 ± 1°C on Trypticase-soy-agar) of 2.2 ± 0.2^5 × 10^6 spores/mg and 1.5 ± 0.2 × 10^6 spores/mg, respectively. Its LC-50's were: 1.6 ng/mm^2 (95% CI: 1.4–1.8) against 1-day-old larvae of *Trichoplusia ni* (bioassay of Ignoffo et al. 1977); 680 ng/ml (95% CI: 560–820 ng/ml; slope 1.62 ± 0.10) at 24 hr against 3rd-instar larvae of *Aedes aegypti*; and 600 IU/mg (compared to the IPS of 1000 IU/mg) at 24 hr for 4th-instar larvae of *Ae. aegypti* and *Culex pipiens quinquefasciatus* (Ignoffo and Couch 1980, unpublished).

The 4-day-old (3rd-instar) larvae of *Ae. aegypti* (L.) weighed 983 ± 30-μg/larva and were reared from eggs obtained from a colony maintained at the Gulf Coast Mosquito Research Laboratory (USDA, SEA, AR), Lake Charles, Louisiana, by T. Fukuda. All tests, unless otherwise specified, were conducted at 24 ± 1°C in 8-ounce waxed ice cream cups (8 S-8G, Lily Corp., Toledo, Ohio 43666) containing 50 larvae/cup in a total volume of 100 ml of distilled water (DW)/cup. Larval mortality was the criterion used to determine the effects of the various test variables. All treatments, including the control, were replicated at least 3 times, and some were replicated as many as 8 times. Throughout the tests larvae were fed pulverized Tetramin (Tetra Werke, D-452, Melle, West Germany) daily unless otherwise specified.

TYPE OF WATER AND INSECTICIDAL ACTIVITY

Three tests were conducted to determine the effects of water type on the insecticidal activity of *Bti*. The 1st and 2nd tests each included 4 treatments: (1) *Bti* suspended in DW (pH 6.6 ± 0.1); (2) *Bti* suspended in pond water (PW), (pH 7.7 ± 0.20); (3) DW without *Bti*; and (4) PW without *Bti*. The PW, obtained from a retaining pond on the grounds of the Bio-Control Laboratory, Columbia, MO, contained 0.82 ± 0.06% dry solids (average of 5 samples). Two concentrations of *Bti* were used (0.1 and 1.0 μg/ml) for the 1st and 2nd test, respectively, and larval mortality was recorded at 3 days and 90 min., respectively. The 3rd test (a rate-mortality test) was conducted after we had established that PW did indeed affect the activity of *Bti*. In this test, larvae were exposed to DW or PW containing the following concentrations of *Bti*: 1000, 100, 50, 10, 1, 0.1, or 0.0 μg/ml. Larval mortality was recorded after 1 hr of exposure and each concentration was replicated 4 times.

In the 1st test, 1 hr of exposure to 1.0 μg/ml *Bti* in DW produced larval mortality of 91.5 ± 3.0% compared with 0% mortality for *Bti* suspended in PW. No mortality was observed in the controls. In the 2nd test, exposure to a rate of 0.1 μg/ml of *Bti* in DW caused about 11 times more mortality than *Bti* in PW (80.0 ± 5.3% vs. 7.3 ± 3.5%). The average percent mortalities of the PW and DW controls in this case were 1.3 ± 0.7% and 0.7 ± 0.4%, respectively.

Results of the rate-mortality portion of the 3rd test (Table 1) confirmed earlier results and provided a direct comparison of LC-50 values. The LC-50, 95% CI, and average ± SE of the slope of the line for the PW treatment were 59.7 μg/ml, 69.1 to 80.3 μg/ml and 1.68 ± 0.11, respectively. The LC-50, 95% CI, and average ± SE of the slope of the line for the DW treatments were 0.7 μg/ml, 0.6 to 0.8 μg/ml, and 1.62 ± 0.10, respectively. Thus, on the basis of the average LC-50 values, the activity of *Bti* in PW was reduced about 85 × over that in DW.

Results of the 3rd test also provided us with LT-50 values that could be used to evaluate the effect of the 2 types of water

Table 1. Average percentage mortality of 4-day old larvae of *Ae. aegypti* after 1-hour exposure to concentrations of *Bacillus thuringiensis* var. *israelensis* suspended in either pond or distilled water.

Concentration (μg/ml)	Average percent mortality[a]	
	Pond water	Distilled water
1000	98.0 ± 0.8	100.0
100	61.0 ± 1.9	100.0
50	37.5 ± 4.3	100.0
10	9.5 ± 5.8	99.0 ± 0.5
1	0.0	60.0 ± 2.3
0.1	0.0	3.5 ± 1.0
0.0	0.0	0.0

[a] Average ± SE $\bar{x}$ of 4 replicates/concentration, 50 larvae/replicate.

on the activity of *Bti*. When mosquito larvae were exposed to *Bti* in PW for 24 hr, the LT-50 values were 0.4, 0.8, 1.2, 2.2, and 24 hr for 1000, 500, 100, 50, 10, and 1 μg/ml, respectively. A similar exposure in DW gave LT-50 values of 0.3, 0.4, 0.9, and 6 hr for 100, 10, 1, and 0.1 μg/ml, respectively. Thus, as the concentration of *Bti* decreased from 100 to 1 μg/ml, the effects of PW on activity increased dramatically from a difference of about 3-fold to almost 27-fold.

CONCENTRATION OF POND SEDIMENT AND INSECTICIDAL ACTIVITY

The PW studies indicated that the larvicidal activity of *Bti* might be a function of concentration of the sediment. The PW sediment (PWS), was oven dried at 167°C for 24 hr, milled (Thomas-Wiley Intermediate Mill, Arthur H. Thomas, Philadelphia, PA 19105) and then filtered through a 20-mesh screen. The particle-size distribution of this sample was: > 500 microns, 55%; < 100 microns 24%. The possible effects of PWS on larval mortality were determined using *Bti* at 1.0 μg/ml and PWS in DW at concentrations of 10.0, 5.0, 2.0, 1.0, 0.75, 0.5, 0.1, 0.05, and 0.0%. Four controls (without *Bti*) were also used: DW and DW + PWS at 10.0, 5.0, and 1.0%. Larval mortality was recorded after 3 and 24 hr, and all treatments were replicated 3 times.

Increasing the concentration of PWS resulted in a corresponding decrease in the insecticidal activity of *Bti* (Table 2). There was almost a 45-fold difference in activity (after 3 hr) between PWS concentration of 0.0 and 5.0%. The reduction in activity of *Bti* may be due to *Bti*-crystal binding with organic materials in the sediment, rather than inactivation of *Bti* by PWS *per se*. About one-half of the *Bti* activity was bound (24-hr bioassay) by 2.0% PWS.

Table 2. Effects of concentration of pond water sediment (PWS) on rate and extent of larval mortality of *Ae. aegypti* caused by *Bacillus thuringiensis* var. *israelensis* (*Bti*).

Concentration PWS 1%	Average percent mortality[a]	
	at 3 hours	at 24 hours
	Sediment treated with Bti[b]	
10.00	0.0	9.0 ± 7.0
5.00	2.0 ± 1.0	40.0 ± 18.6
2.00	3.4 ± 2.4	64.0 ± 30.0
1.00	2.0 ± 1.1	85.3 ± 13.7
0.75	2.0 ± 1.0	90.7 ± 8.4
0.50	8.7 ± 4.6	92.7 ± 6.4
0.10	50.0 ± 6.4	100.0
0.05	45.3 ± 15.9	100.0
0.00	91.3 ± 7.7	100.0
	Sediment not treated with Bti	
10.0	0.0	1.1 ± 0.9
5.0	0.0	0.0
1.0	0.0	0.0
0.0	0.0	0.0

[a] Mean ± SE mean; results of 3 replicates; 50 larvae/replicate/treatment.

[b] Bti used at the rate of 1.0 μg/ml.

pH AND INSECTICIDAL ACTIVITY

The effects of pH on the insecticidal activity of *Bti* were tested both in PW and DW. Three pH conditions (adjusted to pH 4.0 ± 0.1, 6.6 ± 0.1, and 10.0 ± 0.1 by the addition of either HCl or NaOH) were used for each water type, and *Bti* was used at 1.0 μg/ml and 0.0 μg/ml for each pH condition. A natural PW control (pH 7.7 ± 0.2) with and without *Bti* was

also included. Thus, each replicate contained 14 treatments (Table 3). Larval mortality was recorded after 3, 24, and 168 hr, and each treatment was replicated 3 times.

Suspensions of *Bti* in DW were not adversely affected by pH (Table 3). Mortalities in distilled water (after 24 hr) averaged 98, 97, and 94% for pH values of 4.0, 6.6, and 10.0, respectively, and less than 1% control mortality was obtained. Similar results were obtained in pond water where mortalities averaged 52, 32, 25, and 43% for pH values of 4.0, 6.6, 7.4 (natural pond water) and 10.0, respectively, with no control mortality. Although not statistically significant, there appears to be less mortality in natural PW than in acidified or alkalized PW.

UV-LIGHT AND INSECTICIDAL ACTIVITY

A simulated sunlight-UV source, previously demonstrated to inactivate entomopathogenic viruses, a bacterium, a fungus and a protozoan was used (Ignoffo et al. 1977). This UV source provided 0.14 m W/cm² (peak 254 nm, range 215–260 nm) and 1.8 m W/cm² (peak 365 nm, range 290–400 nm) of short-wave and long-wave ultraviolet, respectively, at the plane of the bottom of the test cups. An exposure of 24 hr to the simulated-sunlight source is equivalent to about 6 days of exposure to natural sunlight. Aluminum foil-covered cups (protected) and uncovered cups (not protected) with *Bti* (1.0 μg/ml) in DW and PW (water depth of 2.5 cm) were exposed to UV for 24 hr along with the appropriate controls. Larvae were then added to each cup and mortality recorded after 3, 24, and 168 hr. Thus, each replicate contained 9 treatments (Table 4), and each treatment was replicated 3 times.

Water, it is generally believed, will absorb sunlight/UV as well as other short-wave radiation. Jerlov (1950) and Smith and Baker (1979), however, have shown that this is not true, since UV was nearly as effective as visible light in penetrating clear ocean water. In general, about one-half of the surface radiation of sunlight-UV will penetrate clear ocean water to a depth of about 20 ft. Our results with *Bti* suspended in either DW or PW confirmed these observations. *Bti*

Table 3. Effects of pH on the insecticidal activity of *Bacillus thuringiensis* var. *israelensis* suspended in distilled and pond water.

Test conditions[a]	Average percent mortality (hours)[b]		
	3	24	168
	Distilled water		
Bti at pH 4.0	48.7 ± 19.9	98.0 ± 2.0	100.0
Bti at pH 6.6	46.0 ± 22.5	97.3 ± 2.7	98.7 ± 1.3
Bti at pH 10.0	52.0 ± 22.7	94.0 ± 4.0	99.3 ± 0.7
Control pH 4.0	0.0	0.7 ± 0.6	3.3 ± 0.7
Control pH 6.6	0.0	0.0	2.1 ± 0.2
Control pH 10.0	0.0	0.0	2.1 ± 0.2
	Pond water		
Bti at pH 4.0	0.7 ± 0.4	52.0 ± 25.3	75.3 ± 24.7
Bti at pH 6.6	1.4 ± 1.3	32.0 ± 19.7	75.3 ± 19.9
Bti at pH 7.7	0.0	24.7 ± 14.4	50.0 ± 20.1
Bti at pH 10.0	0.0	42.7 ± 20.3	82.7 ± 12.5
Control pH 4.0	0.0	0.0	4.7 ± 3.6
Control pH 6.6	0.0	0.0	24.7 ± 22.2
Control pH 7.7	0.0	0.0	20.7 ± 10.3
Control pH 10.0	0.0	0.0	12.0 ± 7.5

[a] *Bti* used at 1.0 μg/ml.

[b] Mean ± SE $\bar{x}$; results of 3 replicates; 50 larvae/replicate/test condition.

suspended in DW and exposed for 24 hr to UV lost all its activity (3-hr bioassay) for *Ae. aegypti* (Table 4). In contrast, mortality of the *Bti* sample protected from UV and the *Bti* sample not exposed to UV averaged 68.7 and 79.3%, respectively (Table 4). Differences in mortalities between *Bti* exposed to UV and those not exposed to UV after 24 and 168 hr were about 20-fold and 3-fold, respectively. Similar results were obtained with *Bti* suspended in PW except that mortality was not as high due to the negative interaction of *Bti* + PWS. A sediment concentration of about 1% did not prevent UV-inactivation of *Bti*. Thus, results of both the DW and PW studies indicated that UV could be detrimental to field-applied *Bti*.

SALINITY AND INSECTICIDAL ACTIVITY

Preliminary tests indicated that 4-day-old larvae of *Ae. aegypti* would not survive more than 24 hr in salt concentration of 1.0, 2.5, 5.0, 10.0, and 20.0% NaCl. Since no mortality was obtained at 0.5% (until 72 hr), 3 salt concentrations in DW (0.0, 0.1, 0.5%) without and with *Bti* (at 1.0 μg/ml) were used to determine the effects of salinity on the insecticidal activity of *Bti*. Larval mortality was recorded after 3 and 24 hr, and each treatment was replicated 3 times.

Salt concentrations, at which larvae of *Ae. aegypti* will survive at least 24 hr, did not affect the rate or extent of larval mortality caused by *Bti* (Table 5). Thus, we do not anticipate that salinity, especially of fresh-water and brackish-water pools, will reduce the effectiveness of field-applied *Bti*. Our tests, however, do not exclude possible effects of salts on *Bti* used at sub-lethal rates and against salt-tolerant mosquito species that survive salt concentrations of sea water (ca. 3.5% NaCl).

STABILITY OF AQUEOUS SUSPENSIONS

The rate of inactivation and extent of field persistence are both important factors when one is considering the potential field efficacy of a microbial insecticide. We therefore examined the stability of an aqueous suspension of *Bti* at 30°C, a temperature applicable to anticipated field conditions. A total of 24 test tubes were prepared with 10 ml of *Bti* (in DW) at a concentration of 10 μg/ml. Three tubes were used to establish the baseline mortality for 0 day, and the other 21 were stored at 30°C. Then 3 tubes each were removed after 1, 3, 7, 14, 28, 45, 70, and 120 days of storage and tested for insecticidal activity as follows. The content of a tube was added to 90 ml of DW to provide

Table 4. Effects of simulated sunlight-UV on the insecticidal activity of *Bacillus thuringiensis* var. *israelensis* suspended in distilled and pond water.

Test conditions[a]	Average percent mortality (hours)[b]		
	3	24	168
	Distilled water		
Bti not protected from UV	0.0	4.7 ± 2.9	32.0 ± 19.4
Bti protected from UV	68.7 ± 22.7	98.7 ± 1.3	100.0
Bti not exposed to UV	79.2 ± 12.1	95.3 ± 4.7	100.0
UV-exposed control	0.0	0.0	0.7 ± 0.4
Non-UV-exposed control	0.0	0.0	1.4 ± 1.3
	Pond water		
Bti not protected from UV	0.0	0.0	12.7 ± 5.9
Bti protected from UV	0.0	10.0 ± 6.1	52.0 ± 17.3
Bti not exposed to UV	0.0	2.7 ± 1.3	66.0 ± 11.1
UV-exposed control	0.0	0.0	18.7 ± 7.6

[a] *Bti* used at 1.0 μg/ml and bioassayed against 4-day-old larvae of *Aedes aegypti*.

[b] Mean ± SE $\bar{x}$; results of 3 replicates, 50 larvae/replicate/test condition.

Table 5. Effects of salinity on the insecticidal activity of *Bacillus thuringiensis* var. *israelensis* (*Bti*) against 4-day-old larvae of *Ae. aegypti*.

Test conditions[a]	Average percent mortality (hours)[b] 3	24
Bti at 0.5% NaCl	55.3 ± 19.4	98.0 ± 1.2
Bti at 0.1% NaCl	60.0 ± 19.7	98.7 ± 1.3
Bti at 0.0% NaCl	68.7 ± 14.7	100.0
0.5% NaCl	0.0	0.0
0.1% NaCl	0.0	0.0
0.0% NaCl	0.0	0.0

[a] *Bti* used at 1.0 μg/ml.
[b] Mean ± SE $\bar{x}$; results of 3 replicates, 50 larvae/replicate/test condition.

a bioassay concentration of 1 μg/ml in a total volume of 100 ml DW. A DW control was set up each time the activity of *Bti* was tested. Larvae were exposed for 24 hr, then mortality was recorded. Each time period and treatment including the control was replicated 3 times.

No mortality was recorded in any of the controls. The average percentage larval mortalities for the *Bti* treatment at 0, 1, 7, 28, 45, 70, and 120 days were 96.3 ± 1.1, 92.4 ± 1.9, 95.3 ± 2.7, 97.4 ± 1.2, 99.3 ± 0.7, 92.7 ± 0.7, and 88.0 ± 1.2%, respectively. Thus, the insecticidal activity of *Bti* is relatively stable over a 120-day period when it is stored in an aqueous suspension at 30°C. There appeared to be a slight decrease in activity (ca. 10%), however, at the 120-day reading.

POSSIBLE REGROWTH AND INSECTICIDAL ACTIVITY

Since it was possible that regrowth of the suspended *Bti* might result in more insecticidal activity, 4 variables were used to test this possibility: (1) suspension of *Bti* (0.1 μg/ml) in DW incubated at room temperature for 8 days (24 ± 1°C); (2) a freshly prepared suspension of *Bti* (0.1 μg/ml) in DW; (3) a freshly prepared DW control; and (4) an 8-day-old DW control. Larval mortality was recorded after 3 days of exposure, and all treatments were replicated 4 times.

There was little or no difference in the insecticidal activity of the 2 treatments (Table 6). The average percentage mortalities in the freshly prepared and 8-day-old treatments were 63.5 ± 9.1% and 40.5 ± 16.7%, respectively. Larval mortality in the DW water controls averaged less than 2%.

Table 6. Effects of possible regrowth of *Bacillus thuringiensis* var. *israelensis* and presence of *Bti*-killed larvae on mortality of 4-day-old larvae of *Ae. aegypti*.

Treatment	Average percent mortality[a]
Regrowth test	
Freshly prepared suspension of *Bti*	63.5 ± 9.1
8-day-old suspension of *Bti*	40.5 ± 16.7
Bti-*killed larvae test*	
8-day-old suspension of *Bti* without *Bti*-killed larvae	63.0 ± 1.7
8-day-old suspension of *Bti* with *Bti*-killed larvae	98.0 ± 2.0
Controls	
Fresh distilled water	0.0
8-day-old distilled water	1.5 ± 0.5

[a] Mean ± SE $\bar{x}$; results of 4 replicates; 50 larvae/treatment/replicate; mortality recorded at 3 days.

PRESENCE OF FOOD AND LARVAL MORTALITY

Two treatments were used to determine the effect of food on larval mortality: (1) *Bti* (1.0 μg/ml) without food; and (2) *Bti* (1.0 μg/ml) with food (pulverized Tetramin at 50 μg/ml). Larval mortality was recorded after 2, 3, 6, and 9 hr of exposure. Each treatment was replicated 4 times.

There was no difference in mortality between the 2 treatments (with food 99.0 ± 1.1%; without food 89.5 ± 7.0%) after 6 hr of exposure. However, the presence of food did increase the rate of mortality. Larval mortalities of the *Bti* treatment without food after 2 and 3 hr were 6.0 ± 1.3% and 20.0 ± 3.6%, respectively;

mortalities of the treatment with food for the same periods were 16.0 ± 2.5% and 86.5 ± 8.2%, respectively. The presence of food evidently stimulated feeding and thus increased the probability that the larvae would pick up a lethal dose of *Bti*. If this is true, then the rate of mortality would be influenced even more by exposure to lower concentrations of *Bti*. The differences we observed for the 6-hr period may not have an impact on field control but would influence interpretation of bioassays.

PRESENCE OF DEAD LARVAE AND LARVAL MORTALITY

Since food could influence mortality and vegetative cells with crystals and spores were microscopically detected in dead larvae, the presence of *Bti*-killed larvae could have an effect on mortality. In our 1st test, we compared 2 different treatments using *Bti* at 0.1 μg/ml. In 1 treatment, all 50 *Bti*-killed larvae and larval debris were removed (fine-sieved) after 8 days at room temperature (24 ± 1°C). Dead larvae and larval debris were not removed in the other treatment. There were 2 control treatments, a freshly prepared DW control and an 8-day-old DW control. Larval mortality was recorded after 3 days. Each treatment was assayed with 50 larvae/200 ml per replicate, and all treatments were replicated 4 times.

The *Bti* plus *Bti*-killed larvae treatment was more effective than the *Bti* treatment with all *Bti*-killed larvae removed (Table 6); average percentage mortalities were 98.0 ± 2.0% and 63.0 ± 1.7%, respectively. Mortalities for the freshly prepared DW control and for the 8-day-old DW control were 0.0% and 1.5 ± 0.5%, respectively.

In a 2nd test, 50 *Bti*-killed larvae or 50 heat-killed larvae were added to an 8-ounce cup containing 100 four-day-old larvae in 200 ml of DW. No dead larvae were added to controls and no food other than dead larvae was added to any of the treatments. Larval mortality was recorded after 24 and 72 hr. Each treatment was replicated 3 times using 100 larvae/replicate.

A significant number of larvae were killed by feeding on *Bti*-killed larvae (Table 7); average mortalities were 84.7 ± 9.8% and 97.0 ± 1.0% after 24 and 72 hr, respectively. Mortalities for the heat-killed larvae were 0 and 0.3% after 24 and 72 hr. Control mortality averaged 1.0 ± 0.6 and 1.0 ± 0.6% after 24 and 72 hr, respectively.

In the 3rd test, the combined effects of *Bti* (concentration of 0.1 μg/ml) suspended in PW and the presence of *Bti*-killed larvae were evaluated. The 4 treatments were: (1) a suspension of *Bti* and *Bti*-killed larvae; (2) a suspension of *Bti* and heat-killed larvae; (3) 50 heat-killed larvae; and (4) a PW control. Larval mortality was recorded after 24 and 72 hr. Each treatment was replicated 3 times with 50 four-day-old larvae/200 ml PW per replicate.

The combination of suspended *Bti* and *Bti*-killed larvae was 3 to 4 × more effective than the combination of *Bti* and heat-killed larvae (98.7 ± 1.3% vs. 29.3 ± 3.5%, respectively) (Table 7). Mortalities of the heat-killed control and the un-

Table 7. Effects of presence of larvae killed by either *Bacillus thuringiensis* var. *israelensis* or hot water on mortality of 4-day-old larvae of *Ae. aegypti*.

Treatment[a]	Average percent mortality[a]	
	Distilled water[b]	Pond water[c]
Israelensis-killed larvae	84.7 ± 9.8	—
Heat-killed larvae	0.0	2.0 ± 1.2
Israelensis[d] + *israelensis*-killed larvae	—	98.7 ± 1.3
Israelensis[d] + heat-killed larvae	—	29.3 ± 3.5
Control	1.0 ± 0.6	0.0

[a] Average of 3 replicates ± SE $\bar{x}$; mortality recorded after 24 hours of exposure.
[b] 100 4-day-old larvae/treatment/replicate.
[c] 50 4-day-old larvae/treatment/replicate.
[d] 0.1 μg/ml of *israelensis*.

treated control were 2.0 ± 1.2% and 0%, respectively.

DEPLETION OF ACTIVITY

The possibility that feeding larvae may deplete an introduced inoculum was examined in 3 tests. In test one, nine 1000-ml beakers were filled (3 beakers prepared on each of 3 consecutive days) with 500 ml of *Bti* (concentration of 1.0 μg/ml) in DW. Similar beakers without *Bti* were used as controls. A baseline value for activity was obtained by placing 50 larvae in each beaker containing *Bti*, removing them after 2 hr, and recording the number of dead and living larvae. The beakers were then placed at 30°C, and the procedure was repeated after 1, 2, 3, 6, and 12 days of storage.

The results of the 1st test were as follows: there was no mortality in any of the controls. The average percentage mortality due to *Bti* at 0 day was 96.3 ± 1.8%; mortalities at 1, 2, 3, 6, and 12 days were 94.9 ± 1.0, 62.5 ± 6.1, 20.4 ± 6.7, 4.2 ± 1.4, and 0.9 ± 0.2%, respectively. Thus, less than 1% of the total activity was still present after 12 days and 6 serial exposures of larvae to the original *Bti* suspension.

Test two was conducted to eliminate the possible influence of storage (12 days at 30°C) on depletion; thus, mortality was recorded 3 hr after four-day-old larvae were exposed to a *Bti* suspension (1 μg/ml). Then all dead and living larvae were removed and replaced with another group of living larvae. This sequence was repeated twice, each time with both 50 larvae/cup and 100 larvae/cup. Each treatment was replicated 4 times. Test three was made to evaluate the effect of larval density on depletion (not replicated). Larval density was varied to provide 25, 50, 200, and 400 larvae/100 ml, using *Bti* at 1.0 μg/ml; mortality was recorded after 3 hr of exposure.

The results of the 2nd and 3rd tests confirmed the depletion of inoculum. With 100 larvae/cup, larval mortalities after the 1st, 2nd, and 3rd serial exposures averaged 74.3 ± 4.8, 13.3 ± 4.6, and 0.3 ± 0.2%, respectively. With 50 larvae/cup, mortalities averaged 71.0 ± 4.2, 4.5 ± 2.6, and 1.1 ± 1.0% for the 1st, 2nd, and 3rd serial exposure, respectively. Thus, the combined results of the 3 tests indicated that only 12% of the original activity was present at the 2nd exposure and only 1% was available at the 3rd serial exposure. Mortalities at densities of 25, 50, 200, and 400 larvae/100 ml were 84, 78, 26, and 11%, respectively.

SETTLING OF ACTIVE INGREDIENT

The extent of settling of *Bti* in solution was examined by placing 500 ml of a suspension (1 μg/ml) in a graduated cylinder to provide a 29.3-cm column of DW and *Bti*. After 1 hr, 100 ml of the suspension were carefully siphoned from the top 5.9-cm layer. Also, 100 ml were carefully siphoned from the bottom 5.9 cm of the column. Each sample was then bioassayed against larvae of *Ae. aegypti*. A replicate consisted of 3 treatments: (1) 100 ml of an untreated control, (2) the top 100 ml layer, and (3) the bottom 100 ml layer. Each of the 3 treatments was replicated 8 times.

Suspensions of *Bti* settled rapidly in still water (Table 8). After 1 hr, the difference in larval mortality between the top and bottom sample was more than 50-fold. The difference in mortality, however, was less than 4-fold after 3 hr, and there was no difference after 18 hr. Thus, although there was significant settling of *Bti* in only

Table 8. Average percent mortality of 4-day-old larvae of *Ae. aegypti* exposed to the top and bottom layers of a 29.3-cm column of distilled water containing *Bacillus thuringiensis* var. *israelensis*.[a]

Hours of larval exposure	Top 5.9 cm of cylinder	Bottom 5.9 cm of cylinder
1.0	0.8 ± 0.4	41.8 ± 9.9
1.5	3.2 ± 1.0	74.0 ± 3.5
2.0	8.2 ± 1.3	87.0 ± 2.2
2.5	17.0 ± 1.6	93.5 ± 1.5
3.0	28.2 ± 4.4	98.5 ± 0.5
18.5	98.8 ± 0.7	100.0

[a] Concentration of 1 μg/ml; mean ± SE $\bar{x}$ of 8 replicates, 50 larvae/replicate.

1 hr, there was still sufficient activity present in the top layer to provide nearly 100% mortality when larvae were exposed for 24 hr.

GENERAL DISCUSSION

Var. *israelensis* was effective in the laboratory against larvae of *Ae. aegypti* and has good potential for field control of species of *Aedes* and other mosquitoes. Anticipated field rates (extrapolated from laboratory results reported by Goldberg and Margalit 1977, de Barjac 1978, Garcia and Desrochers 1979, and herein) are estimated at between 1/4 and 2 kg/ha. This is the equivalent of ca. 0.4 to 2.9 billion LC-50 units/ha (based on our *Ae. aegypti* bioassay) and 0.2 to 1.2 billion IU/ha (based on 600 IU/mg bioassayed against *Ae. aegypti*). However, our laboratory studies indicated that the kind of water, pond sediment, sunlight-UV, and settling in water might reduce available *Bti*. Thus, additional research on these facts may be needed to increase field effectiveness. Field stability, at least within our test parameters, did not appear to be a major problem, but preliminary evidence indicated that the shelf stability of a dry wettable powder preparation was reduced at 50°C (½ life of ca. 8 to 10 days), at 30°C (½ life ca. 50 days) and thus may require further investigation. On the other hand, although *Bti* did not reproduce after application to water, additional control may be anticipated by larvae feeding on *Bti*-killed larvae that contain vegetative cells with crystals and spores of var. *israelensis*. Thus, all available laboratory evidence indicates that var. *israelensis* is a promising control agent for mosquitoes, and future efforts should be made to improve activity, stability, formulations, and application procedures.

References Cited

de Barjac, H. 1978. Un nouveau candidat a la lutte biologique contre les moustiques: *Bacillus thuringiensis* var. *israelensis*. Entomophaga 23:309–319.

Briggs, J. D. 1963. Symposium on culture procedures for arthropod vectors and their biological control agents. W.H.O. Bull. 31:1–622.

Briggs, J. D. 1975. Biological Regulation of Vectors. A Conference Report: U.S. Dept. of Health, Education, and Welfare, Easton, Maryland. Publication NIH, 77–1180, 174 pp.

Garcia, R. and B. Desrochers. 1979. Toxicity of *Bacillus thuringiensis* var. *israelensis* to some California mosquitoes under different conditions. Mosquito News 39:541–544.

Goldberg, L. J. and J. Margalit. 1977. A bacterial spore demonstrating rapid larvicidal activity against *Anopheles sergentii, Uranotaenia unguiculata, Culex univitattus, Aedes aegypti* and *Culex pipiens*. Mosquito News 37:355–358.

Ignoffo, C. M. 1965. Production, identification, and standardization of insect viral pathogens. Entomophaga 10:29–40.

Ignoffo, C. M. 1967. Possibilities of mass-producing insect pathogens. Proceedings International Colloquium Insect Pathol. and Microbial Control, Wageningen, the Netherlands, Sept. 5–10, 1966:91–117. North Holland Publ. Co., Amsterdam, 1967.

Ignoffo, C. M., C. Garcia, M. J. Kroha and T. Fukuda. 1980. Susceptibility of *Aedes aegypti* to four varieties of *Bacillus thuringiensis*. Mosquito News 40:290–291.

Ignoffo, C. M., D. L. Hostetter, R. E. Pinnell and C. Garcia. 1970. Relative susceptibility of six soybean caterpillars to a standard preparation of *Bacillus thuringiensis* var. *kurstaki*. J. Econ. Entomol. 70:60–63.

Ignoffo, C. M., D. L. Hostetter, P. P. Sikorowski, G. Sutter and W. M. Brooks. 1977. Inactivation of representative species of entompathogenic viruses, a bacterium, fungus, and protozoan by an ultraviolet light source. Environ. Entomol. 6:411–415.

Jerlov, N. G. 1950. Ultra-violet radiation in the sea. Nature 166:111–112.

Laird, M. 1971. Microbial control of arthropods of medical importance. In: "Microbial Control of Insects and Mites." (Eds. H. D. Burges and N. W. Hussey) Academic Press: London, New York. 86 pp.

Smith, R. C. and K. S. Baker. 1979. Penetration of UV-B and biologically effective dose-rates in natural waters. Photochem. Photobiol. 29:311–323.

Steinhaus, E. A. and D. W. Jenkins. 1960. Biological control of insects of medical importance. Armed Forces Institute of Pathology. Tech. Report, American Institute of Biological Sciences, Washington, DC, 144 pp.