EFFECTS OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* ON *AEDES TAENIORHYNCHUS* AND SOME NON-TARGET ORGANISMS IN THE SALT MARSH

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ABSTRACT. Laboratory and field studies were conducted with *Bacillus thuringiensis* var. *israelensis* (BTI) to determine its effect upon *Aedes taeniorhynchus* and non-target organisms in the salt marsh. Toxicity tests indicated that salt-marsh mud reduced the activity of BTI. With corrections, BTI killed over 99% of the mosquito larvae in the field at concentrations of 4.5 IU/ml and above. Out of 39 species collected prior to treatment, only *Notonecta indica* showed a significant decrease in population. However, this genus is known to fly from deteriorating habitats.

INTRODUCTION

The endotoxin of *Bacillus thuringiensis* var. *israelensis* (serotype H-14) is extremely toxic to a wide variety of mosquito larvae under laboratory and field conditions (Goldberg and Margalit 1977, de Barjac 1978, Garcia and Desrochers 1979, Sinegre et al. 1979b, Garcia et al. 1980b, Mulla et al. 1980). However, only 2 laboratory studies are known to have dealt with its effect upon *Aedes taeniorhynchus,* our district's most vexacious species (Mulla et al. 1980, Van Essen and Hembree 1980). For future control work, we needed to know this species susceptibility to *B. thuringiensis* var. *israelensis* (BTI) in its breeding habitat, the upper salt marsh. A preliminary field trial in brackish, mud-bottom holes had shown no significant mortality at a rate double that which had killed all of the larvae in a preliminary toxicity test. Therefore toxicity tests were run with mud to determine its effect on BTI.

Numerous studies have examined the effect of BTI on non-target organisms in the laboratory (Sinegre et al. 1979a), under simulated field conditions (Garcia et al. 1980a, Miura et al. 1980), and in the field (Dejoux 1979, Colbo and Undeen 1980, Miura et al. 1980, Mulla et al. 1980). However, only one other has in-

vestigated this relationship in a salt marsh, and it contained no mangroves (Garcia and Desrochers 1980).

METHODS AND MATERIALS

Biochem Products of Montcharin, Delaware, supplied the BTI. The wettable powder had a reference number of 666 PM50 and 3,000 International units *Aedes aegypti*/mg (IU/mg). Concentrations in IU/ml equal 0.333 ppm for this product.

The 2 toxicity tests used 3rd and 4th instar larvae of *Ae. taeniorhynchus* reared from eggs supplied by the Insects Affecting Man and Animals Laboratory, U.S. Dept. of Agriculture, Gainesville, Florida. Fresh serial dilutions were made by pipetting at least 10 ml of BTI suspension into demineralized water. Each dilution was stirred magnetically for a minimum of 15 min. The first test used 15 larvae in 118 ml plastic soufflé cups containing 100 ml solutions of 0.2% rock salt. The test consisted of 3 sets each with a control plus 5 treatments at 0.015, 0.030, 0.060, 0.12, and 0.24 IU/ml. Two sets contained about 10 ml of mud from a local salt marsh while another was run without mud. The second test was expanded to 3 mud and 3 no-mud replicates with 20 larvae per cup. The concentrations of the mud cups were increased (Table 2) so that an LD_{50} could be obtained. Also, since the mud had increased the salinity to 0.7–1.0% (mean 0.8%) by the end of the first test, no rock salt was used in the mud cups. In both tests, the water was lightly dusted with a 1:1:1 food mixture of brewer's yeast, liver powder, and hog supplement. The cups were kept between 29°C and 30°C.

Twenty-four hours later the live larvae were counted, and the water quality was determined. From the dose versus mortality data, a simple linear regression equation was calculated. An inverse prediction for the LD_{50} and its 95% confidence interval was then calculated from this equation (Zar 1974).

The first field experiment was run on September 26 and 27, 1980, in 4 proximate water holes just north of Wiggins Pass, Florida. They contained seawater that had spilled over the beach berm on a series of very high tides. Each water hole was measured for surface area and mean depth to estimate its volume. Ten dips were taken in each of the control holes and 20 dips in the holes designated to be treated. Dips were made without regard to mosquito abundance while walking on a predetermined course that transversed the entire hole. Each dip contained 150 ml of water. A meter stick was placed in Hole 3 to record any change in the water level. To monitor flooding by the next high tide, small buoyant sticks were placed evenly on a sand bank sloping 30° into Hole 3.

The water holes were treated with a well-suspended mixture of BTI from a plastic, pump-up, hand sprayer. At 30 lbs pressure, it delivered 8.8 ml/second or 10.6×10^4 IU/second. Holes 1 and 2 were controls; Hole 3 received 9.0 IU/ml; Hole 4 received 3.0 IU/ml. Twenty-four hr later, Holes 1 and 3 were measured and dipped again; Holes 2 and 4 had dried up.

The second field experiment was run on October 2 and 3 in a small brackish pond located about 2 mi. northwest of Isle of Capri. Measurements were taken to estimate the surface area and volume. A meter stick was placed in the water to reference any level change. Twelve and 14 in. strips of galvanized roof flashing were sealed and riveted at the ends to form enclosures with an area of 1.7 m^2. Five enclosures were pushed into the mud to prevent an interchange of water.

Dips of 190 ml each were taken before treatment and 23 hr later. Since *Ae. taeniorhynchus* reacts to changes in light, movement of water, and sound (Nielsen and Nielsen 1953), 20 dips were taken quietly without casting a shadow within the enclosure. Although dipping disturbed nearby larvae, the distance and time between successive dips largely minimized this effect. All portions of the enclosure were sampled with equal inten-

sity. Thirty dips were also taken in the remainder of the pond in the same manner as the first field experiment. These methods are considered random sampling since all larvae had an equal and independent chance of being collected.

All but 2 control enclosures received applications of BTI with a calibrated hand sprayer. They received concentrations of 4.5, 9.0, and 13.5 IU/ml from a well-suspended mixture of 15,000 IU/ml. The remainder of the pond (65 m^3) received a dosage of 9.0 IU/ml from a mixture of 3.75×10^5 IU/ml.

Non-target species were sampled before treatment and 23 hr later with a D-shaped dip net having 1.0 ml mesh. Three replicate samples, each comprised of 5 consistent sweeps, were taken in the saltgrass, *Distichlis spicata*. A sweeping method was chosen since it could quickly and effectively collect large numbers of invertebrates. Although it has numerous drawbacks for estimating terrestrial insect populations (DeLong 1932), few of them apply for aquatic insect populations.

The non-target taxa were identified by keys in: Herring (1951), Chapman (1958), and Gonsoulin (1973) for the Hemiptera; Young (1954) for the Coleoptera; Kaston (1948) for the Araneae; Borror and DeLong (1971) for the Homoptera; and Edmonson (1959) for the Diptera, Ephemeroptera, and Lepidoptera.

Nonparametric statistical methods were used on the data of the 2nd field experiment to avoid untenable assumptions about the distribution or variance of the populations. Chi-square analysis tested the null hypothesis that BTI had no significant effect on larval survival. The number of larvae which survived treatment (Table 2) was compared to the number expected to survive based on the mean survival rate in the control areas. The Mann-Whitney statistic tested the one-tailed, null hypothesis that each non-target population was greater or equal after treatment (23 hr) than before treatment at the 0.05 significance level.

RESULTS

Table 1 shows the mean percent survival from the replicates of the 2nd toxicity test. In the first test, the mud cups had no significant mortality; and the no-mud cups had mortalities similar to those of the 2nd test. At the end of the 2nd test, the salinity was 0.2% (no mud) and 0.5–0.8% with a mean of 0.55% (mud); the pH was 6.4 (no mud) and 7.1–7.3 (mud); the dissolved oxygen was 5.8–6.1 ppm (no mud) and 3.9–5.2 ppm with a mean of 4.4 ppm (mud).

Table 1. The mean survival from three replicates of *Aedes taeniorhynchus* larvae treated with various concentrations of BTI with mud present or absent in the second toxicity test.

No Mud		Mud	
Concentration IU/ml	Mean survival % ± SD	Concentration IU/ml	Mean survival % ± SD
0.24	0 ± 0	9.4	0 ± 0
0.12	38 ± 23	3.8	0 ± 0
0.060	77 ± 23	1.5	62 ± 13
0.030	87 ± 3	0.60	93 ± 3
0.015	97 ± 3	0.24	87 ± 3
0	93 ± 6	0	93 ± 8

The dose versus mortality data for the 2nd test fit simple linear regressions with $Y=-433X+99.9$ ($r=+0.94$) for the no-mud replicates and $Y=-26.3X + 101$ ($r=+0.98$) for the mud replicates. The LD_{50} of the former regression was 0.12 IU/ml with the 95% confidence interval between 0.014 and 0.290. The LD_{50} of the latter regression was 1.9 IU/ml with the 95% confidence interval between 0.91 and 2.3. The latter regression also had an LD_{90} of approximately 3.5 IU/ml.

For the duration of the first field experiment, Holes 1 and 3 retained water. The initial mean depth and volume of Hole 1 was 8 cm and 0.17 m^3, respectively. It had a mean and standard deviation (SD) of 34± 25 *Ae. taeniorhynchus* larvae per dip, 3.2% salinity, and a thick coverage of *D. spicata*. The larvae

totaled an estimated 38,000. Hole 3 had an initial mean depth and volume of 14 cm and 4.9 m^3, respectively. The salinity was 3.3%. It had a mean and SD of 58± 64 *Ae. taeniorhynchus* larvae per dip for an estimated total of 1.8 million. Thick *D. spicata* grew throughout the hole except for a central 4.0 × 1.5 m area with a sandy-mud bottom. The larvae were more abundant in this open section than amongst the grass. White mangrove, *Laguncularia racemosa,* grew around the edge of the hole and *Batis maritima,* saltwort, was abundant at one end.

Hole 3 changed considerably in 24 hr. The displacement of the bottom 3 sticks on the sand slope indicated that a high tide, due 14 hr after treatment, had raised the water 3–4 cm above the initial level. Since that time, seepage had dropped the water to 9 cm below the initial level. Hole 3 then had an area and volume of 17.5 m^2 and 1.4 m^3 respectively. The mean number of mosquito larvae collected per dip was 2.7±3.8 SD for an estimated total of 25,000. The mortality was 95% uncorrected or 99% when corrected for the 72% decline in water volume. The area had a rotten odor from all the dead mosquitoes, which were pink and white. All observed non-target species, including *Mesovelia* sp., a water treader, *Uca* sp., a fiddler crab, *Tropisternus* sp., a water scavenger beetle, and other water beetles, were alive.

Hole 1 had dried to a few pockets of water whose size allowed only 3 isolated dips of 600 larvae. The lack of water precluded an accurate estimate of larval abundance and a comparable control. However, no dead larvae were observed in the dips, and all appeared alive in the wet spots.

The 2nd field experiment was run in a pond about 50 m from the Gulf of Mexico. The elliptic surface area was estimated at 330 m^2. The mean depth was 20.3 cm for an estimated volume of 67 m^3. The usually shaded pond was located in a grove of *Casuarina* sp., Australian pine, with *Rhizophora mangle*, red mangrove, and *L. racemosa* around the edges. Emergent *D. spicata* covered one-third of the pond. The remainder of the pond was open water with a mud bottom. The pond contained about 6.7 million *Ae. taeniorhynchus* larvae, which occurred mostly in the open area. The salinity was 0.5% on the first day and 0.2% on the 2nd day. The water volumes in enclosures 1 through 5 were 170, 220, 350, 350 and 310 liters, respectively. Between 16 and 24 hr after treatment, rain raised the water level 4 cm. The water volumes increased 69 liters in each enclosure and about 15 m^3 in the pond.

Table 2 summarizes the dip counts and percentages of each instar. The chi-square statistic for the 3 enclosures and pond equaled 80.49 so that the null hypothesis was rejected with $P < 0.001$. Thus, a very significant difference existed between larval survival in the treated areas and the control areas.

Sixty-one non-target species were collected in the second field experiment

Table 2. A summary of the data from the second field experiment before treatment with BTI and 23 hours later.

Area	Concentration IU/ml	Before: Mean # larvae per dip ± SD	Before: Instar: % of each	After: Mean # larvae and pupae per dip ± SD	After: Instar: % of each	Survival %
Encl. 1	0	50.9 ± 50.5	2:20/3:80	39 ± 32.5	2:5/3:10/4:84/P:1	77
Encl. 2	13.5	48.9 ± 46.4	2:15/3:80/4:5	0.050 ± 0.22	4:100	0.1
Encl. 3	9.0	25.0 ± 38.9	3:85/4:15	0.10 ± 0.45	P:100	0.4
Encl. 4	0	17.1 ± 15.4	2:10/3:80/4:10	11.5 ± 9.0	2:5/3:30/4:61/P:4	67
Encl. 5	4.5	19.0 ± 11.6	2:20/3:75/4:5	0 ± 0	—	0
Pond	9.0	19.6 ± 16.9	2:15/3:80/4:5	0.10 ± 0.37	P:100	0.5

(Table 3). Eighty-four percent of these taxa were Coleoptera, Hemiptera, Odonata and Araneae. Thirty-nine species were collected before treatment, and 48 were collected 23 hr later. Almost twice as many Coleoptera species were collected after treatment as before, but the other major taxa were about equally distributed. A tadpole was the only vertebrate observed or collected.

Five species comprised 71% of the individuals collected. Two of them were highly dominant in the aquatic community. There were 572 individuals of *Belostoma testaceum*, a giant water bug and 388 of a larval *Odontomyia* sp., a soldier fly.

Table 3. The non-target taxa collected in the second field experiment before treatment with BTI and 23 hours later.

Taxa	Before	After	Taxa	Before	After
Anura—Toads and Frogs[a]					
Tadpole sp. 1	0	1			
Araneae—Spiders					
Araneae sp. 2	3	1	*Pirata* sp. 1	22	32
Araneae sp. 5	1	0	Tetragnathidae sp. 1	1	0
Araneae sp. 6	0	1	Tetragnathidae sp. 2	3	1
Araneae sp. 8	0	1	Tetragnathidae sp. 4	0	1
Lycosidae spp.	39	22	Tetragnathidae sp. 5	0	1
Coleoptera—Beetles					
Acilius sp. 1[a]	16	31	*Haliplus* sp. 1[a]	0	1
Anodocheilus exiguus	0	29	*Hydrochus* sp. 1	0	1
Berosus infuscatus	0	1	*Illybius* sp. 1[a]	1	0
Berosus sp. 1[a]	4	2	*Rhantus calidus*[a]	1	5
Coleoptera sp. 1	0	1	*Scirtes* sp. 1[a]	0	33
Copelatus caelatipennis	0	3	*Thermonectus basillaris*	15	15
Donacia sp. 1	1	0	*Tropisternus* sp. 1[a]	3	5
Dytiscidae sp. 3[a]	0	1	*Tropisternus lateralis nimbatus*	0	3
Haliplus confluentus	2	3			
Diptera—Flies[a]					
Ceratopogonidae sp. 1	0	1	Emphydridae sp. 3	0	2
Chironomidae sp. 1	1	2	*Odontomyia* sp. 1	130	258
Ephemeroptera—Mayflies[a]					
Baetidae sp. 1	0	9			
Hemiptera—True Bugs					
Belostoma lutarium	2	0	*Mesovelia amoena*	15	30
Belostoma sp. 1	0	2	*Mesovelia mulsanti*	32	45
Belostoma testaceum	254	318	*Mesovelia* spp.[b]	0	4
Buenoa elegans	6	0	*Microvelia hinei*	2	9
Gelastocoris oculatus[a]	1	1	*Notonecta indica*	3	0
Hebrus buenoi	0	2	Reduviidae sp. 1	1	0
Hebrus concinnus	0	2	Reduviidae sp. 2	1	0
Limnogonus hesione	3	5	*Trichocorixa verticalis verticalis*	0	2
Merragata brevis	0	1			
Homoptera—Leaf hoppers, etc.					
Cercopidae sp. 1	4	1	Delphacidae sp. 1	153	178
Cixidae sp. 1	2	0			

Table 3. (Continued).

Taxa	Before	After	Taxa	Before	After
			Lepidoptera—Butterflies and Moths[a]		
Lepidoptera sp. 4	3	0			
			Odonata—Dragonflies and Damselflies[a]		
Agrionidae spp.[b]	3	2	*Erythrodiplax* sp. 1	1	0
Anax sp. 2	58	21	*Ishnura* sp. 1	11	6
Anax sp. 5	13	7	Libellulidae sp. 1	1	0
Coenagrionidae sp. 1	13	5	Libellulidae spp.[b]	4	3
Coenagrionidae spp.[b]	0	3	*Tramea* sp. 1	17	10
Enallagma sp. 1	2	4			

[a] Immatures only.
[b] Taxa too small or damaged to identify but not considered distinct from other species.

The next most abundant aquatic species was *Anax* sp. 2, a darner, with 79 individuals. Delphacidae sp. 1, a delphacid planthopper that lived among the emergent *D. spicata*, was the most numerous terrestrial species with 331 individuals. The semi-aquatic *Mesovelia mulsanti* was also abundant with 87 individuals.

The size of all populations except *Notonecta indica* (Hemiptera: Notonectidae) was statistically the same or greater after treatment as before treatment. This species was collected once in each before-treatment replicate and not at all afterwards.

DISCUSSION

Only Van Essen and Hembree (1980) conducted toxicity tests on *Ae. taeniorhynchus* with some data referenced to international units. Without this reference, data reported in ppm or spore counts are difficult to compare since the potency is unknown or unreliable (Van Essen and Hembree 1980). Their LD_{50} of 0.256 IU/ml was higher than the LD_{50} of 0.12 IU/ml in this study. However, this study was run at a higher temperature, which has been shown to increase the susceptibility of the larvae to BTI (Sinegre et al. 1979b).

The higher LD_{50} for larvae tested with mud indicates that the mud had a strong adverse effect on the activity of BTI. Prasertphon and Rishikesh (1979) reported that mud drastically reduced its activity against *Anopheles gambiae* Giles larvae. The toxicity tests with mud gave a better estimate of the dosage needed in the muddy areas of the salt marsh and may be a preferable method for determining application rates for such areas.

Corrections can be made on the data in Table 2 that would increase the survival in the controls and decrease it in the treated areas. Pupae may be excluded from the survival data since BTI must be ingested for it to act. Pupae do not eat, and late 4th instars eat relatively little (Nielsen and Haeger 1954, Mulla et al 1980). Even with Abbott's correction (Abbott 1925), all the treated areas then had 100% mortality except for 99.9% in the 13.5 IU/ml enclosure. The increased water volume tended to underestimate the survival of larvae by making them less available to dipping. With the pupal correction, this effect was most pronounced on the control areas. It is mitigated by the tendency of 3rd and 4th instars to spend much of their time at the water surface. The very high mortalities indicate that the delayed dilution of BTI had little effect on the results.

With corrections, both field trials achieved over 99% mortality at 4.5 IU/ml and above. This value represents the maximum dosage of BTI that would be needed to attain close to 100% mortality

in our salt marshes with these larvae. With an approximate LD_{90} of 3.5 IU/ml from the toxicity test with mud, this dosage may be approaching the minimum control dosage.

The salt marsh was a harsh environment in which BTI proved very effective. It withstood water level fluctuations, mud, tannin, and salinity. High tannin levels, such as occur in mangrove marshes, were believed to inhibit *B. thuringiensis* var. *thuringiensis* (Garcia and Desrochers 1979). Although salinity disrupts many biological control agents, it does not appear to affect the activity of BTI, as Sinegre et al. (1979b) also noted.

As often observed on other occasions, *Ae. taeniorhynchus* congregated in certain parts of the habitats. Nielsen and Nielsen (1953) also observed the clustering of *Ae. taeniorhynchus* in open areas. In contrast, the other aquatic fauna, mostly mosquito-larvae predators (Purcell 1980), were much more abundant in the vegetated areas. In the enclosures, the larvae crowded around the edges. This aggregated distribution caused the means of the dip counts to have large standard deviations.

The backswimmer *Notonecta indica* comprised the only non-target population which declined significantly. Since they are well documented as efficient mosquito-larvae predators (Hinman 1934, Bay 1967, Lee 1967, Ellis and Borden 1970, Toth and Chew 1972), they may have died from eating larvae that had ingested BTI. However, other studies have shown that such exposure does not affect them (Garcia et al. 1980a, Miura et al. 1980). When the mosquito larvae died, they more likely flew in search of a habitat with a better food supply. Others have reported such migrations (Blatchley 1926, Toth and Chew 1972). Besides the use of BTI for simuliid (black fly) control, numerous other studies have found no acute toxic effects to aquatic organisms (Weiser and Vankova 1978, Dejoux 1979, Singre et al. 1979a, Colbo and Undeen 1980, Garcia and Desrochers 1980, Miura et al. 1980, Mulla et al. 1980). Two studies observed adverse effects only to midges of the Dixidae, Ceratopogonidae and Chironomidae (Garcia and Goldberg 1977, Garcia et al. 1980a). Thus tests to date on its effects to aquatic species are encouraging.

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