TOXICITY OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* TO SOME CALIFORNIA MOSQUITOES UNDER DIFFERENT CONDITIONS

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ABSTRACT. *Bacillus thuringiensis* var. *israelensis* (WHO/CCBC 1897) was shown to produce 100% mortality at a dosage of 1×10^5 bacilli/ml in laboratory studies against six species (in 3 genera) of mosquito larvae commonly found in California. Tests were conducted in water from natural breeding sources which included treehole and brackish sites. Neither different water qualities nor full sun exposure apprared to diminish the activity if the pathogen under the conditions of these tests.

Bacillus thuringiensis var. *israelensis* was originally isolated from the milieu of a mosquito breeding pool in a drying river bed in Israel by Goldberg and Margalit (1977). These workers designated the isolate as ONR 60A and demonstrated that it was rapidly toxic to the larvae of *Anopheles sergentii, Uranotaenia unguiculata, Culex univittatus, Cx. pipiens* and *Aedes aegypti* in laboratory studies. They also showed that the pathogenic activity was due to an endotoxin which showed no significant loss in activity after a heat shock of 20 min at 60°C, exposure to ultraviolet (2537A°) or lyophilization. ONR 60A has also been designated as WHO/CCBC 1897 by the World Health Organization. A recent analysis by de Barjac (1978) identified ONR 60A/WHO CCBC 1897 as a new serotype (H14) of *Bacillus thuringiensis* and gave it the varietal name of *israelensis.*

The studies reported here evaluated the toxicity of this pathogen in a variety of different aquatic habitats against the following 6 species of mosquitoes common in northern California: *Ae. sierrensis, Ae. dorsalis, Cx. pipiens* complex, *Cx. tarsalis, Culiseta incidens* and *Cs. inornata.*

METHODS AND MATERIALS

The stock preparation of *Bacillus thuringiensis* var. *israelensis* used in all tests reported here was prepared by Leonard Goldberg of the Naval Bioscience Research Laboratory, University of California, Alameda, California. The bacteria were cultured on nutrient agar and harvested in a water slurry which was used either directly as the stock preparation or was lyophilized and resuspended with water (Goldberg and Margalit 1977). The bacterial count at harvest was 10^{10} bacilli/ml which was used as the base for the 10-fold serial dilutions used in the tests.

The mosquito larvae used in these experiments were obtained and tested from the following sources. The western treehole mosquito *Ae. sierrensis* was collected from rot holes in the coast live oak *Quercus agrifolia* in Marin County or from rot holes in the stumpage of the California black oak *Q. kelloggi* found in Blodgett Experimental Forest, El Dorado County. One test was also conducted on the 1st instar progeny of wild caught adults from Blodgett Forest. A total of 8 treehole populations were used, each maintained in their own treehole water along with a small amount of oak debris. The reared 1st instar progeny were tested in laboratory medium made from the boiled, decanted water extract of yeast and liver extract[1] added to an equal volume of tap water.

Ae. dorsalis in the 2nd and 3rd instar and 4th instar *Cs. inornata* were collected from brackish water potholes and a tidal creek, respectively, in the Petaluma salt

[1] 2.5 g Brewer's yeast and 4 g liver extract concentrate (1:20) in liter water.

marsh, Sonoma County. Total salt concentration was less than 1% with a pH of approximately 7 at both sources during the collection period of June, 1977 (Metzger, pers. comm.).

Cs. incidens was collected and tested in water from large artificial containers filled by rain runoff at pH's of 7.0 to 7.4. Larvae of *Cx. pipiens* complex were collected in association with populations of *Cs. incidens.* The only larvae used from a colonized source were *Cx. tarsalis* in the 2nd and 3rd instars received from Monica Asman, University of California, Berkeley. They were reared and tested in water containing a small amount of their food source of ground rabbit chow pellets.

Tests were conducted both outside and inside the laboratory in uncapped glass specimen jars in a final test volume of 200 ml. The test concentration used ranged from 1×10^4 to 1×10^6 bacilli/ml with a few exceptions to 1×10^3 bacilli/ml. All test concentrations were replicated 3 times and corrected for control mortality by Abbott's method (Abbott 1925).

The majority of tests conducted outdoors were in a wood-roofed screen enclosure which provided complete shade during the entire observation periods. Ambient temperatures ranged between 6° and 21°C during the test periods.

One set of tests was conducted indoors and outdoors simultaneously to evaluate the impact of direct sunlight on the pathogen activity. A bright clear day was selected (September 30, 1978) and the outdoor test containers were positioned to receive full sunlight between 10:30 a.m. and 5:30 p.m. P.S.T. for an exposure period of 7 hours. They were then returned to the laboratory for the remaining observation period of 96 hr. To prevent excess heat build-up due to the direct exposure to bright sunlight the test and control containers were placed in enamelled pans partially filled with tap water. Ambient temperatures during the course of the outdoor exposure ranged from 17°C to 25° ±.5°C and the water temperatures ranged from 14.5° to 31° ±1°C. The ambient temperatures for this and all indoor tests remained a fairly constant 22° ±2°C.

RESULTS AND DISCUSSION

As indicated in Table 1, larvae treated in all water sources regardless of stage of development died when exposed to a pathogen concentration of 10^5 bacilli/ml or greater. Among the *Ae. sierrensis* populations tested at a concentration of 10^4 bacilli/ml, mortality was highly variable ranging from 0 to 92%. Water from the 1st treehole showed the highest mortality as well as the lowest recorded pH at 5.5. Mortality in treehole water with more basic pH recordings ranged from 0 to 62% and showed no positive correlation with either an increasing or decreasing value. The excellent efficacy of this pathogen in an assortment of different treehole waters, which can be considered harsh conditions as far as water quality is concerned, is extremely encouraging. Tests conducted several years earlier with another strain of *B. thuringiensis* var. *thuringiensis,* (BA-068) which was known to be particularly active against *Aedes* spp. failed to cause observed mortality differences between treated and untreated treehole populations in Marin County (Reeves 1970, Reeves and Garcia 1971, and Reeves and Garcia unpubl.). The tests with BA-068 were conducted in treeholes in the same geographic area as some of the treehole water and larval sources tested in this study. It was believed that the tannins and possibly other factors present in the treehole water inhibited the pathogenic action of BA-068.

Cs. inornata and *Ae. dorsalis* larvae tested in brackish water demonstrated a 40% and 100% mortality respectively at a concentration of 10^4 bacilli/ml. There was no indication that brackish water from the Petaluma salt marsh affected the efficacy of the pathogen, however, at the time of the collections the salt concentration was relatively low due to the spring inflows of fresh water. Larvae of the laboratory strain of *Cx. tarsalis* and the wild caught larvae of *Ae. dorsalis* were the only species

Table 1. Observed larval mortality among selected species of mosquitoes when exposed to varying concentrations of *Bacillus thuringiensis* var *israelensis* (WHO/CCBC 1897).

Species instar(s)	Source	Water pH	No. dead larvae/total 10^6	10^5	10^4	10^3
Ae. sierrensis						
1st	eggs	7.4	90/90	90/90	41/90	
2nd, 4th	live oak	5.5	13/13	13/13	12/13	
4th	live oak	6.4	30/30	30/30	13/29	
2nd, 4th	live oak	7.9	13/13	13/13	2/12	
4th	live oak	8.0	30/30	30/30	14/30	
2nd, 4th	live oak	8.1	12/12	12/12	0/12	
4th	live oak	8.4	30/30	30/30	19/30	
2nd, 3rd	black oak	7.4	30/30	30/30	3/30	
2nd, 3rd	black oak	8.0	30/30	29/29	0/29	
Ae. dorsalis						
2nd, 3rd	salt marsh	7.0	30/30	30/30	30/30	
Cx. pipiens						
4th	barrel	7.4		15/15	1/14	0/15
2nd	barrel	7.4		30/30	9/30	0/30
Cx. tarsalis						
2nd, 3rd	lab strain	7.0	30/30	30/30	30/30	
Cs. inornata						
4th	creek	7.0	30/30	30/30	12/30	
Cs. incidens						
2nd, 3rd	barrel	—	28/28	26/26	0/29	
4th	barrel	—	17/17	18/18	0/16	
4th	barrel	—	26/26	25/25	11/26	
4th	barrel	7.4		30/30	1/30	1/30
2nd	barrel	7.4		12/12	1/12	2/12

to show complete mortality through the 10^4 concentration. Whether this susceptibility was due to some physiological difference inherent in the species or due to some physical factor of the aquatic environment will require further testing. Mortality at 10^4 tended to be highly variable among the different species and water systems under test (Table 1).

Populations of *Cx. tarsalis* and *Ae. sierrensis* were used for the comparative experiments which were run indoors and outdoors simultaneously. In the case of *Cx. tarsalis* 100% mortality was observed both indoors and outdoors within 2 hr for the 10^6 and 10^5 concentrations and within 24 hr for the 10^4 concentration (Table 2). In the tests with *Ae. sierrensis*, 100% mor-

Table 2. Larval mortality of *Ae. sierrensis* and *Cx. tarsalis* exposed to *Bacillus thuringiensis* var *israelensis* (WHO/CCBC 1897) under indoor and outdoor conditions.

Species instar(s)	Source	Conditions	No. dead larvae/total 10^6	10^5	10^4
Ae. sierrensis					
2nd, 3rd	black oak #7	outdoors[1]	30/30	30/30	22/30
2nd, 3rd	black oak #7	indoors	30/30	30/30	3/30
3rd, 3rd	black oak #8	outdoors	30/30	29/29	23/29
2nd, 3rd	black oak #8	indoors	30/30	29/29	0/29
Cx. tarsalis					
2nd, 3rd	lab strain	outdoors	30/30	30/30	30/30
2nd, 3rd	lab strain	indoors	30/30	30/30	30/30

[1] Outdoors received 7 hr. exposure to direct sunlight.

tality was seen both indoors and outdoors within 2 hr at 10^6 and within 24 hr at the 10^5 concentration. Mortality was not complete for this species at the 10^4 concentration in either location. For the groups remaining indoors throughout the test the mortality was 0 and 10% whereas the groups exposed to the 7 hr of sunlight had mortality rates of 73% and 79% (Table 2). The higher temperatures of the outdoor tests during the 7 hr period of sun exposure may account for the observed differences. Water temperatures in the sun exposed group ranged from 31°C at about 12 noon down to 14.5°C in the late afternoon at the end of the exposure. The temperatures for this outdoor group averaged over 28°C for the first 4 hours of the experiment, whereas the temperature in the laboratory for the same period was 22±2°C.

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