LARVICIDAL ACTIVITY OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* AGAINST *AEDES TRISERIATUS* IN TREEHOLE AND TIRE HABITATS[1]

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ABSTRACT. *Bacillus thuringiensis* var. *israelenis (Bti)* was tested as a larvicide against the mosquito, *Aedes triseriatus*, under laboratory and field conditions. In distilled water and exposed tire water, *Bti* was about 4 times more effective than it was in beech treehole water, oak treehole water, and shaded tire water. The LD-50 for *Bti* in the treehole water and shaded tire water was 0.4 ppm and the LD-99 was 3.0 ppm. Concentrations from 1.0 to 10.0 ppm *Bti* caused high mortality to mosquito larvae in all breeding habitats tested. A bioassay using laboratory-reared fourth instar *Ae. triseriatus* showed that under field conditions *Bti* loses most of its larvicidal activity in treeholes and tires within 3 to 5 days. Small scale field trials with *Bti* using a Hudson sprayer in a tire yard reduced *Aedes triseriatus* breeding by 98%.

Since its discovery by Goldberg and Margalit (1977), *Bacillus thuringiensis* var. *israelensis (Bti)* has been extensively tested to determine its potential for mosquito control. It is now considered one of the most promising biological insecticides for controlling mosquito breeding in a wide range of situations. *Bacillus thuringiensis* var. *israelensis* demonstrates an extremely rapid larvicidal activity against a variety of mosquito species. The LT-50 against *Aedes aegypti* (Linnaeus) of 12.2 ± 1.1 min at a concentration of 90 ug/ml (Ignoffo et al. 1980) is similar to more conventional chemical control agents. Goldberg and Margalit (1977) showed the range of mosquito species affected by testing representative species from 4 genera. Later work by Garcia and DesRochers (1979) showed its toxic effect on 6 mosquito species common to northern California.

One of the major advantages of *Bti* is its extreme specificity for dipterans of the suborder Nematocera. Tests conducted with diverse aquatic insects, crustaceans, amphibians, fish and mammals have shown that these non-target organisms are unaffected (Garcia et al. 1980). Under experimental conditions, the toxic action of *Bti* is largely unaffected at pH values between 4.0 and 10.0 and salt concentrations below 0.5% (Ignoffo et al. 1981). It is unaffected by temperatures as high as 60°C applied for periods of up to 20 minutes. Additionally, *Bti* is considered to be more stable in the presence of ultraviolet light than other entomopathogens such as fungi, protozoa and viruses (Ignoffo et al. 1977).

Our studies with *Bti* focused on *Ae. triseriatus* (Say), a common species of mosquito found east of the Rocky Mountains (Zavortink 1972). This mosquito is the major vector of LaCrosse encephalitis in the midwest (Thompson et al. 1972) and a possible vector of dog heartworm, *Dirofilaria immitis* (Intermill 1973). Though most frequently associated with treeholes, *Ae. triseriatus* may also be found in a wide variety of artificial containers, such as tires, rain gutters, tin cans and bird baths. Due to the inaccessibility of the primary larval habitat and the reluctance of the adults to leave deep wooded areas, no practical means of control are available at present.

This study compares the larvicidal activity of *Bti* against *Ae. triseriatus* in distilled water and water from 4 natural larval habitats. The range of lethal concen-

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trations determined in the laboratory was then tested against natural populations of *Ae. triseriatus* in treeholes and in tires. A bioassay system was used in the field to quantify the temporal decrease in larvicidal activity of *Bti* against *Ae. triseriatus*. Preliminary field control trials with *Bti* were conducted in a tire yard producing large numbers of *Ae. triseriatus*.

MATERIALS AND METHODS

The *Bti* used in the study was a wettable powder produced by Abbott Laboratories Inc. (North Chicago, IL 60064) designated ABG-6108-II. The powder was manufacturer-rated at 2000 International *Aedes aegypti* Toxic Units per milligram. Stock solutions of 1000 ppm were made by suspending one gram of the powder in 1000 ml of distilled water. The larvae used in the laboratory tests and the field bioassay were *Ae. triseriatus* WALTON strain, from a colony maintained by the Vector Biology Laboratory at the University of Notre Dame.

The study was divided into 4 parts. The first stage consisted of laboratory tests to determine the LD-50 and LD-99 of *Bti* in distilled water, oak treehole water, and beech treehole water. In addition, water was tested from tires in direct sunlight and from tires shaded by vegetation. All water from natural habitats was first filtered through a nylon mesh to remove large particulates, as well as any larvae already present. Fourth instar *Ae. triseriatus* larvae were subjected to concentrations of *Bti* between 0.01 and 10.0 ppm in each type of water at 21°C. For each of 3 replicates at each concentration, 20 fourth instar *Ae. triseriatus* were placed in 200 ml of water in a 400 ml paper cup. Percent mortality was determined after 24 hours of exposure to the insecticide.

In the second stage of the experiment, the *Bti* was tested in the field. Eighteen oak treeholes, 18 beech treeholes, and 18 tires containing *Ae. triseriatus* were chosen from sites in St. Joseph County, Indiana. Experiments spanned a 3-day period. On the first day, all of the water in each treehole or tire was removed using a hand pump and a turkey baster. The water volume and number of third and fourth instar *Ae. triseriatus* were determined in the field. All the water and the larvae were returned to the treehole or tire immediately after the counting was completed. After a 24 hr period during which most of the sediment had settled, a predetermined quantity of *Bti* stock solution (1000 ppm) was added to produce the desired concentration. The concentration assigned to each treehole or tire was determined by ranking the treeholes or tires by the volume of water in each and then dividing them into 3 size classes. One treehole or tire from each size class was randomly selected for testing at each concentration in order to prevent any bias due to size differences. Afer 24 hr of exposure to the *Bti*, the water was once again removed using the same method and the number of surviving *Ae. triseriatus* larvae was determined.

The third stage of the experiment consisted of a field bioassay to determine the effectiveness of *Bti* over time. Ten oak treeholes, 10 beech treeholes, and 10 tires were used. The volume of water in each treehole or tire was determined as previously described. After a 24 hr reequilibration period, *Bti* was added to produce a concentration of 10.0 ppm in 6 of the 10 treeholes or tires selected. The other 4 treeholes or tires served as controls.

The sampling system in the bioassay utilized a modification of the Horsfall cage. Each rectangular cage measured 2.5 × 2.5 × 13 cm. The ends and 2 of the sides were made of clear plastic. The other 2 sides were covered with a double layer of nylon mesh. The mesh allowed water to flow in and out of the cage, but prevented mosquito larvae from entering or escaping. Access to the inside of the cage was through a 1.2 cm hole in the top which was closed with a tight fitting cork. One modified Horsfall cage containing 15 *Ae. triseriatus* larvae was lowered into each treehole or tire. The bottom of the cage was allowed to rest in the sediment at the bottom of the treehole and at least 3.0

cm of the cage remained above the water surface. After 24 hr of exposure, the number of survivors and the number of dead larvae inside the cage were determined by rinsing the cage in a pan of distilled water. A new group of 15 larvae was tested each day over a 7 day period.

For the field control study, an auto salvage yard with a heavy population of *Ae. triseriatus* was utilized. Three separate tire piles were chosen. Six tires were randomly selected from each of the piles and the number of larvae in each tire was determined. After a 24 hr reequilibration period, a 1000 ppm solution of *Bti* was broadcast as a fine spray over the tires of the first tire pile using a Hudson sprayer (11.3 liter capacity). For the second tire pile a concentrated stream of *Bti* solution was sprayed directly into the tires using the Hudson sprayer. The third tire pile served as a control and received no application of *Bti*. After an exposure period of 24 hr, the water was once again removed from the tires and the number of survivors was determined.

RESULTS

An LD-50 of 0.4 ppm and an LD-99 of 3.0 ppm were obtained for the oak treehole, the beech treehole, and the shaded tire water tested in the laboratory (Table 1). An LD-50 of 0.1 ppm and an LD-99 of 1.0 ppm were found for the distilled water and the exposed tire water.

The LD-50 and LD-99 values determined for beech treeholes in the field were 4 times higher than those determined in the lab (Table 2). The LD-50 was 1.7 ppm and the LD-99 was 12.0 ppm. The results of the oak and tire field studies more closely paralleled the laboratory values. Even at the lowest concentration tested, 1.0 ppm, there was no significant drop in toxicity in the oak treeholes or tires. The lowered percent mortality in tires at 5.0 ppm is due to one trial which had a mortality of only 80%.

Though only the number of survivors was recorded during the field study, many dead larvae were observed in the treated treeholes and tires. The only other animals which appeared to be affected were Chironomidae larvae. Large numbers of dead chironomid larvae were observed floating at the surface of all treated tires. Though the manufacturer claims that *Bti* is effective against Ceratopogonidae larvae, live ceratopogonids were observed in treeholes treated at concentrations as high as 50.0 ppm. Helodidae beetle larvae, common inhabitants of treeholes, were likewise unaffected.

Bacillus thuringiensis var. *israelensis* has a limited effective lifespan under natural conditions (Fig. 1). The bioassay indicated

Table 1. Observed larval mortality and standard error of the mean of *Aedes triseriatus* when exposed to *Bacillus thuringiensis* var. *israelensis* in distilled water, beech treehole water, oak treehole water and two types of tire water under laboratory conditions.

Concentration	Percent mortality				
(ppm)	Distilled	Beech	Oak	Exposed tire	Shaded tire
Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
0.01	3.3 ± 1.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
0.05	15.0 ± 8.7	0.0 ± 0.0	0.0 ± 0.0	10.0 ± 5.0	0.0 ± 0.0
0.1	31.7 ± 6.7	0.0 ± 0.0	0.0 ± 0.0	26.7 ± 1.7	3.3 ± 3.3
0.2	80.0 ± 2.9	25.0 ± 5.8	18.3 ± 6.0	68.3 ± 3.3	21.7 ± 3.3
0.4	83.3 ± 3.3	63.3 ± 8.8	65.0 ± 2.9	98.3 ± 1.7	65.0 ± 2.9
0.7	100.0 ± 0.0	75.0 ± 2.9	78.3 ± 6.7	100.0 ± 0.0	83.3 ± 4.4
1.0	98.3 ± 1.7	81.7 ± 4.4	88.3 ± 4.4	100.0 ± 0.0	86.7 ± 4.4
10.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
50.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0

Table 2. Observed larval mortality and standard error of the mean of *Aedes triseriatus* when exposed to *Bacillus thuringiensis* var. *israelensis* in beech treeholes, oak treeholes, and tires.

Concentration (ppm)	Percent mortality		
	Beech	Oak	Tires
Control	12.7 ± 12.7	6.0 ± 6.0	2.0 ± 2.0
1.0	46.0 ± 11.1	85.3 ± 7.8	100.0 ± 0.0
5.0	75.7 ± 15.4	96.3 ± 3.7	93.0 ± 6.5
10.0	98.3 ± 1.7	98.3 ± 1.7	99.0 ± 1.0
50.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
100.0	97.3 ± 1.8	98.7 ± 1.3	100.0 ± 0.0

that after 6 or 7 days the toxic effects of *Bti* were negligible. Though the variations in beech treeholes and tires were found to be minimal, oak treeholes showed a marked variability in the effectivensss of *Bti* over time. This variability was most pronounced during the second and third day of the study. No data were collected from the tires on the fifth day because of limited access to the tire yard on weekends. On several days squirrels, which frequently use treeholes as a source of water, disrupted several of the Horsfall cages. Data values were then averaged for the remaining intact cages.

Application of 1000 ppm *Bti* solution by both broadcast method and direct spraying in tires produced 98% mortality. The controls showed only 2% mortality.

DISCUSSION

Nearly identical LD-50 and LD-99 values were found for the oak treehole water, the beech treehole water, and the shaded tire water. The nearly constant toxicity of *Bti* when applied to these varying water types attests to the insecticide's stability under a range of chemical conditions. The greater toxicity of *Bti* in distilled water and exposed tire water is consistent with the findings of Ignoffo et al. (1981). They found that the activity of

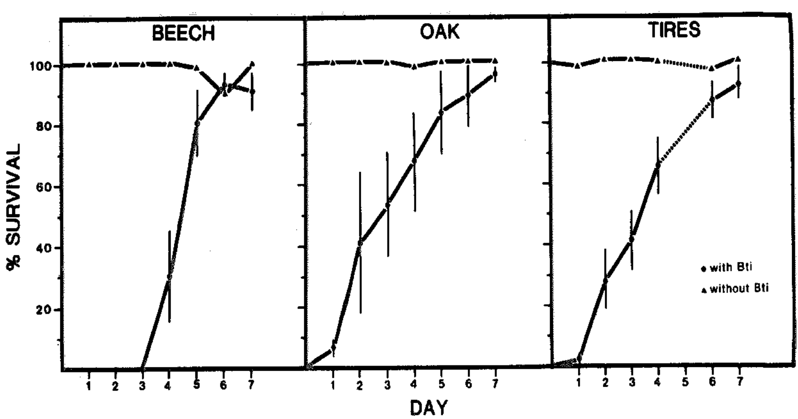

Fig. 1. Effectiveness of *Bacillus thuringiensis* var. *israelensis* (at a concentration of 10 ppm) against *Aedes triseriatus* over a 7 day period in beech treeholes, oak treeholes and tires. Vertical lines represent standard errors about the means and the dotted lines indicate a single day of missing values.

Bti is reduced by sediment levels as low as 2%.

Bacillus thuringiensis var. *israelensis* proved nearly as effective in the field as it had been in the laboratory. Only the beech treeholes differed significantly from the laboratory results. The reason for this difference is unknown.

The results of the field bioassay reflect the extreme variability of treeholes. This variability was especially evident in the oak treeholes. The contents of the oak treeholes ranged from nearly clear water to a thick mud. If the toxic action of *Bti* in treeholes is consistent with its effects in other aquatic systems (Ignoffo et al. 1981), then it is likely that treeholes with the greatest sediment levels would be least affected by *Bti*.

The modified Horsfall cages used during the field bioassay proved to be highly effective. They were easily removed and replaced. Furthermore, larval counts were simple to perform. The mesh on the sides of the cages not only prevented larvae from escaping, but also effectively hindered predation.

The results indicate that *Bti* is a potentially valuable means of control against *Aedes triseriatus*. The LD-50 and LD-99 values occur at concentrations which can easily be achieved by either the addition of powder or solution. Difficulties will surely be encountered in delivering *Bti* to treeholes because of their inaccessible nature, and therefore, its use may be limited to control operations in foci of LaCrosse encephalitis activity. However, as the results of the field control trials indicate, conventional means of spraying with *Bti* can easily be used to treat tires. Recent epidemiological evidence suggests that the majority of human cases of LaCrosse encephalitis in the midwest U.S. are associated with *Aedes triseriatus* breeding in artificial containers, notably discarded automobile tires (DeFoliart and Lisitza 1980). The application of *Bti*, an insecticide which is safe for humans, might provide an alternative to conventional chemical control.

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