THE EFFECT OF TEMPERATURE, LARVAL AGE, AND SPECIES OF MOSQUITO ON THE ACTIVITY OF AN ISOLATE OF *BACILLUS THURINGIENSIS* VAR. *DARMSTADIENSIS* TOXIC FOR MOSQUITO LARVAE

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ABSTRACT. An isolate of *Bacillus thuringiensis* var. *darmstadiensis* (H-10) with preferential toxicity for mosquitoes was evaluated in the laboratory against *Culex quinquefasciatus, Aedes aegypti, Anopheles albimanus* and *An. quadrimaculatus*; the LC_{50} values for 2nd instar larvae were 0.41, 3.96, 4.62 and 5.38 viable spores $\times 10^4$/ml, respectively. There was a strong positive correlation between temperature and larvicidal activity against fourth instar *Cx. quinquefasciatus* exposed to 3.46×10^4 viable spores/ml at 18, 24 and 31°C. Second instar *Cx. quinquefasciatus* and *Ae. aegypti* were approximately 10× more susceptible to the spore-crystal suspension than were fourth instar larvae.

Several strains of *Bacillus thuringiensis* Berliner that are active against lepidopterous larvae have demonstrated moderate activity against mosquitoes (Reeves and Garcia 1971, Hall et al. 1977, Panbangred et al. 1979). Conversely, isolates of *B. thuringiensis* var. *israelensis* (H-14) show remarkable larvicidal activity against mosquitoes and reduced or virtually no activity against Lepidoptera (de Barjac 1978, Tyrell et al. 1979, Ignoffo et al. 1981). Similar findings were reported by Padua et al. (1980) for two isolates of *B. thuringiensis* var. *darmstadiensis* (H-10) tested against three culicine mosquitoes and two species of Lepidoptera. This paper presents information on the larvicidal activity of the 73-E-10-16 isolate of *Bacillus thuringiensis* var. *darmstadiensis* (H-10) against culicine and anopheline mosquitoes, as well as the effect of larval age and temperature on activity.

METHODS AND MATERIALS

A culture of the 73-E-10-16 isolate of *B. thuringiensis* (H-10) obtained from Dr. Michio Ohba (Institute of Biological Control, Kyushu University, Fukuoka, Japan) was transferred to and grown on tryptose blood agar in petri plates at 37°C for 4 to 5 days. Sporulating colonies were scraped from the plates and suspended in distilled water and stored at 4°C until used. Spore counts of the suspensions were made from a pasteurized sample using the standard pour plate procedure. Immediately prior to bioassay, serial dilutions of the stock suspension were made. Forty-eight-hour 2nd instar *Culex quinquefasciatus* Say, *Aedes aegypti* (L.), *Anopheles quadrimaculatus* Say and *An. albimanus* Wiedemann obtained from colonies maintained at the Insects Affecting Man and Animals Research Laboratory were exposed to seven concentrations of the spore-crystal inclusion suspension in 100 ml of well water in waxed paper cups. The concentrations were selected such that at least two concentrations produced mortality above and two produced mortality below the 50% mortality point. The bioassay procedure utilized 20 larvae/cup, 3 cups/concentration and a control for each replicate. Three replications were run over several weeks. During the exposure period, cups were maintained at 27°C by placing them on thermostatically controlled heat tapes. For the first 24 hr of exposure, larvae were not fed. Approximately 10 mg of larval diet (hog supplement) were added to each cup during the second 24 hr of exposure. Preliminary trials indicated that mortality after 24 hr of exposure was considerably less than at 48 hr. Thereafter, all tests were terminated and mortality was assessed after 48 hr. In order to determine the effect of larval age on mortality, early 4th instar *Cx. quinquefasciatus* and *Ae. aegypti* were exposed to seven concentrations of the spore-crystal suspension under the same conditions utilized for the 2nd instar larvae. The influence of temperature was studied with early 4th instar *Cx. quinquefasciatus* exposed to 3.4×10^4 viable spores/ml of *B. thuringiensis* (H-10) at 17.7, 24.3 and 31.0°C. The number of replicates and exposure conditions were identical to those for the 2nd instar larvae except that exposures were made in temperature-controlled cabinets.

The mortality data for all but the temperature study were analyzed with log-probit analysis after correction for control mortality with Abbott's formula. The temperature mortality data were analyzed using linear regression analysis (general linear models procedure).

RESULTS

The comparative susceptibility of 2nd instars of the four species is graphically presented in

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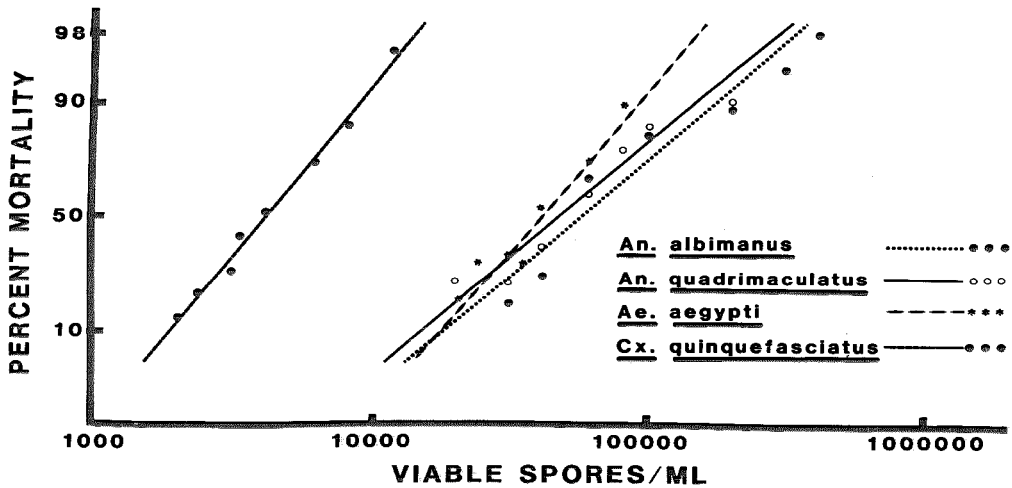

Fig. 1. Probit analysis of *Bacillus thuringiensis* (H-10) against second instar *Culex quinquefasciatus, Aedes aegypti, Anopheles quadrimaculatus* and *An. albimanus.*

Fig. 1. The LC_{50} and LC_{95} values and their respective 95% confidence intervals are presented in Table 1. *Culex quinquefasciatus* was significantly more susceptible than the other three species. The LC_{50} values for *Ae. aegypti* and the two anophelines were not significantly different from one another.

Second instar *Cx. quinquefasciatus* and *Ae. aegypti* were ca. 10 times more susceptible to *B. thuringiensis* (H-10) than were 4th instar larvae (Figs. 1 and 2). The LC_{50} and LC_{95} values and their respective 95% confidence intervals are presented in Table 1.

The effect of temperature on mortality of *Cx. quinquefasciatus* exposed to *B. thuringiensis* (H-10) is presented in Table 2. A direct and positive relationship between mortality and temperature was observed (R^2= 0.99; F = 163.63; MSE = 0.527; PR>F = 0.0001).

DISCUSSION

Only *Cx. quinquefasciatus* displayed a high level of susceptibility to the 73-E-10-16 isolate. The other three species tested would require a prohibitive amount of inoculum for effective

Table 1. LC_{50} and LC_{95} values for *Culex quinquefasciatus, Aedes aegypti, Anopheles albimanus* and *An. quadrimaculatus* larvae exposed to *Bacillus thuringiensis* (H-10) (27°C).

Species	LC	Concentration and 95% fiducial limits (× 10^4 viable spores/ml)	
		Second instar	Fourth instar
Culex quinquefasciatus	50	0.41	3.40
		0.39–0.43	2.46– 4.47
	95	1.10	16.95
		1.00– 1.24	11.01– 37.83
Aedes aegypti	50	3.96	39.65
		3.51– 4.52	33.84– 46.07
	95	11.47	117.19
		8.70–18.64	91.34–174.01
Anopheles quadrimaculatus	50	4.62	—
		3.82– 5.39	—
	95	20.23	—
		15.57–30.05	—
Anopheles albimanus	50	5.38	—
		4.09– 6.83	—
	95	22.41	—
		15.57–42.17	—

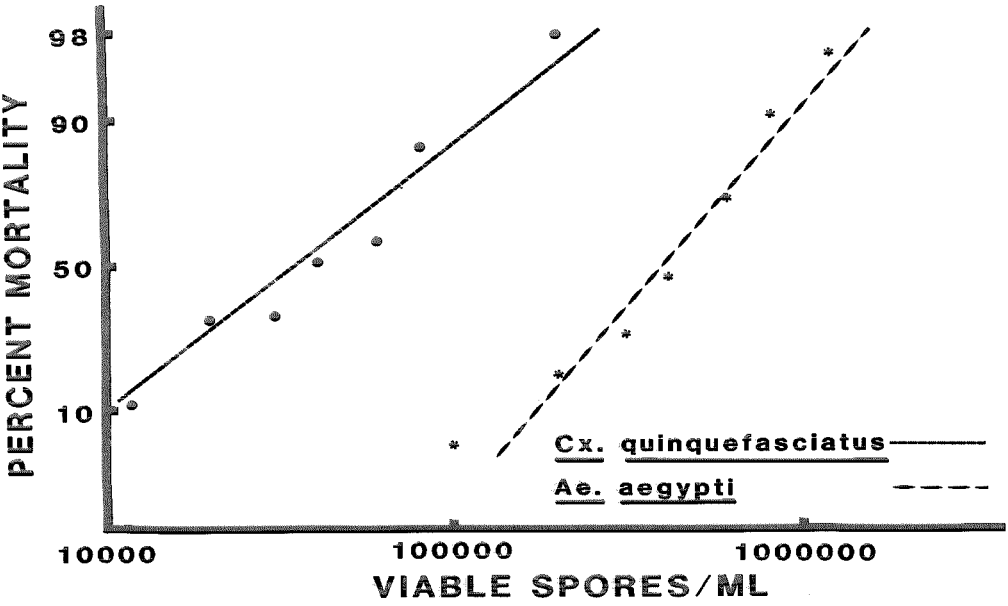

Fig. 2. Probit analyis of *Bacillus thuringiensis* (H-10) against forth instar *Culex quinquefasciatus* and *Aedes aegypti*.

control under practical conditions. Padua et al. (1980) reported similar susceptibility levels: the 73-E-10-16 isolate displayed good activity against *Culex* spp., reduced activity against *Ae. aegypti* and very low activity against two species of Lepidoptera. Previous investigations using other isolates of *B. thuringiensis* (H-10) revealed little or no activity against mosquito larvae (Hall et al. 1977, Larget and de Bargac 1981).

The larvicidal activity of the serotype 10 isolates of *B. thuringiensis* against mosquitoes is probably due only to the spherical parasporal crystalline inclusion. Previous studies with other serotypes against mosquitoes (Panbangred et al. 1979, Tyrell et al. 1979) and black flies (Lacey et al. 1978) indicated that the moieties responsible for larvicidal activity were found in the crystalline inclusions. Despite the long period of post-exposure incubation prior to the expression of maximum mortality in black fly larvae, Lacey and Federici (1979) found no involvement of the spore in the pathogenesis of *B. thuringiensis* var. *kenyae*. The slower acting nature of the δ-endotoxin from this isolate compared with that of the H-14 isolates of *B. thuringiensis* (48 hr vs. <24 hr) is not totally unexpected. Ignoffo et al. (1980) observed a marked increase in LT50 between *B. thuringiensis* (H-14) and other varieties. Similarly, Larget and de Barjac (1981) reported increased mortality in mosquito larvae between 24 and 48 hr exposures to several varieties of *B. thuringiensis* but not to the H-14 serotype. Padua et al. (1980) confirmed the absence of β-exotoxin in the 73-E-10-16 isolate, a toxin produced by certain varieties of *B. thuringiensis* during vegetative growth that also is a biocide of dipterans.

The positive relationship between temperature and mortality and the negative correlation between larval age and mortality reported here

Table 2. Effect of temperature on mortality of 4th instar *Culex quinquefasciatus* exposed to 3.4×10^4 viable spores/ml of *Bacillus thuringiensis* (H-10)

	Percent mortality ± S.E.	
Temperature (°C)	Treated	Control*
17.7±0.4	35.0±2.9 a	0 a
24.3±0.7	55.0±5.8 b	0 a
31.0±1.1	83.3±1.7 c	1.7 a

* Control mortality was significantly different from treatments at the 0.001 level. Means in the same column followed by the same letter are not significantly different at the 0.05 level.

with *B. thuringiensis* (H-10) and *Cx. quinquefasciatus* has been observed with other serotypes of *B. thuringiensis* bioassayed against mosquitoes (Panbangred et al. 1979, Van Essen and Hembree 1980, Wraight et al. 1981) and black fly larvae (Lacey et al. 1978, Molloy et al. 1981). A rise in temperature may increase mortality through several different mechanisms acting singly or in concert, e.g., increased feeding rate and hence increased uptake of toxic crystals, and/or accelerated dissolution, activation and absorption of the toxic moieties. Negative correlation of larval age with mortality is a phenomenon observed with most chemical and microbial control agents. The exact mechanism of reduced susceptibility of older instars to *B. thuringiensis* is not completely known. In addition to lower physiological susceptibility, older instars were exposed to less toxin/mg of body weight than were younger instars.

Despite the rather elevated larvicidal activity of the 73-E-10-16 isolate against *Cx. quinquefasciatus*, it is still considerably less active than the *B. thuringiensis* (H-14) isolates against the other test species.

In a concurrent study conducted in this laboratory (Lacey and Singer 1982), the LC_{50} of the IPS-78 standard of *B. thuringiensis* H-14 against 4th instar *Aedes aegypti* was 0.2028 ppm. The number of viable spores/mg of IPS-78 was 4.24×10^7. The number of spores/ml of bioassay water at the LC_{50} concentration of IPS-78 was 8.60×10^3. In this light IPS-78 is clearly more active against *Ae. aegypti* than the 73-E-10-16 isolate. On the other hand, *Cx. quinquefasciatus* is approximately 10× more susceptible than *Ae. aegypti* to the 73-E-10-16 isolate and, as generally reported in the literature, only half as susceptible to *B. thuringiensis* H-14 (Tyrell et al. 1979, Ali et al. 1981). Comparison of activity based on spore counts, however, is tenuous at best. The media employed and procedures for cultivation and harvesting of *B. thuringiensis* may exert more influence on toxicity than relative number of spores and their accompanying parasporal crystalline inclusions (Dulmage 1981). The use of spore counts for comparative purposes in our study is only meant to indicate possible trends.

Based on our results and those reported elsewhere in the literature, other serotypes of *B. thuringiensis* appear to be poor substitutes for serotype H-14. However, serotypes with selective activity, such as those isolates of *B. thuringiensis* (H-10) with preferential activity toward certain culicine mosquitoes, may be desirable when it is advantageous to protect nematocerous predators, such as *Toxorhynchites* spp., that may be harmed by the H-14 serotype.

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FAILURE OF AN INSECT ELECTROCUTING DEVICE TO REDUCE MOSQUITO BITING[1]

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ABSTRACT. Insect electrocuting devices using ultraviolet light as an attractant were tested for their ability to reduce mosquito biting in backyards. Biting collections were made in 6 adjacent backyards located in South Bend, Indiana. Two of the yards were equipped with electronic insect killing devices, 2 with CDC traps, and 2 had no apparatus. The collections were made on 8 nights from June through August 1982. The insects killed by the electrocuting devices also were collected. Only 3.3% of the 3212 insects killed on an average night were female mosquitoes. Humans in the vicinity of the electrocuting devices were consistently more attractive to mosquitoes than the devices. Even after 11 days of continuous operation, the electrocuting devices failed to reduce the mosquito biting rate.

INTRODUCTION

Electronic insect killing devices using an electrocuting grid and ultraviolet (UV) light as an attractant (commonly sold as Zappers,™, Bugwackers,™, Bug Blasters,™ etc.) frequently are used in an attempt to control pest insects in backyards, campgrounds, swimming pools and other outdoor recreation areas. These devices are advertised on the basis of claims by the manufacturers that they have a "lure range" of a certain distance depending on the power of the UV source, they can clear insects from a certain radius around the trap, and they disrupt the "breeding cycle" of insects. Test data available from some of the manufacturers indicate that, if they are the sole source of light, these devices are useful in reducing indoor house fly populations. Unfortunately, no data are presented on the efficiency of these traps outdoors or their ability to reduce mosquito biting; the purpose for which most of the traps are purchased. This study was designed to evaluate the ability of these devices to reduce mosquito biting in backyard situations.

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MATERIALS AND METHODS

The study was conducted from late June through August 1982 in a suburban neighborhood on the northeast side of South Bend (St. Joseph County), Indiana. The area was known to have moderate to heavy local populations of *Aedes vexans* (Meigen) and *Ae. trivittatus* (Coquillett) (St. Joseph County Mosquito Abatement Program, Unpublished data). The testing was conducted in 6 backyards within the neighborhood. The backyards were adjacent to each other in a straight north to south line. Each backyard was approximately 35 m square. The 6 yards were surrounded on the north, south, and east by similar residential areas. Adjacent to the yards to the west was a small, shallow drainage ditch (ca. 1 m wide) frequently containing water and intermittently producing *Ae. trivittatus*. Immediately to the west of the drainage ditch was a 5 to 15 m wide woodlot running almost the entire length of the backyards. The woodlot consisted of large, mixed deciduous trees (trunk diam. 10–40 cm) and a dense herbaceous understory. To the west and south of the woodlot was a schoolyard consisting of mowed grass. Immediately to the north of the woodlot was an unmowed field with vegetation ranging from 0.5 to 2 m high. The land-