LABORATORY BIOASSAY OF *BACILLUS THURINGIENSIS ISRAELENSIS* AGAINST ALL INSTARS OF *AEDES AEGYPTI* AND *AEDES TAENIORHYNCHUS* LARVAE[1]

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ABSTRACT. Laboratory bioassays of *Bacillus thuringiensis israelensis* (BTI) were conducted against 5 larval age groups of *Aedes aegypti* and *Ae. taeniorhynchus*. *Ae. aegypti* was more susceptible to BTI than *Ae. taeniorhynchus* in all age groups except the 6 hr old one. For 72 hr old larvae, the LD_{50} was 0.36 ppm for *Ae. aegypti* and 0.52 ppm for *Ae. taeniorhynchus* after a 48 hr exposure to BTI. Younger larvae of both species were more susceptible than older larvae. Comparative assays were run with IPS 78 material from the Institut Pasteur and a portion of the data is expressed as relative activity to facilitate comparison with other studies.

INTRODUCTION

The United States Army, in its search for alternative mosquito control methods, has been interested in recent developments in the use of microbial agents. Singer (1973) reported isolates of *Bacillus sphaericus* with a high level of larvicidal activity, and evaluation and development of this prospective biological control agent is underway. Strains of *Bacillus thuringiensis* in widespread use in agriculture have generally demonstrated little prospect for usefulness against mosquitoes, but Hall et al. (1977) reported several strains with a high level of activity in bioassays against 5 species of *Aedes* and *Culex*. A new strain of *B. thuringiensis* was recently found in Israel by Goldberg and Margalit (1977) and was designated *B. thuringiensis israelensis* (serotype H 14) by de Barjac (1978a). Bioassays have been conducted against several species of mosquitoes (Goldberg and Margalit 1977, de Barjac 1978b, de Barjac and Coz 1978, Garcia and Desrochers 1979) and black flies (Undeen and Nagel 1978, Undeen and Berl 1979). The results of these tests are difficult to compare because some are reported in terms of number of colony forming units or number of spores per unit volume and others are reported in terms of weight of formulation per unit volume. Quantity of actual active ingredient has not yet been related to either weight of formulation or to number of colony forming units. De Barjac has performed a very valuable service by providing a standard material that will permit, after comparative tests are run, the reporting of results of dose-response studies in terms of international units. None of the dose-response studies published to date have dealt in detail with the effects of age of the target on susceptibility to the agent.

The objective of the studies reported here was to determine the effect of age of *Aedes aegypti* and *Ae. taeniorhynchus* larvae on their susceptibility to *Bacillus thuringiensis israelensis* (BTI). A portion of the results will be reported as relative activity, compared to the international standard material (IPS 78) supplied by the Institut Pasteur.

MATERIALS AND METHODS

MOSQUITO REARING. Both species of mosquitoes tested in this study were reared under constant conditions of 27°C and 65% RH for adults and 28°C for lar-

[1] Opinions, assertions, or product names contained herein are the private views of the authors and are not to be considered official or as reflecting the views or endorsements of the Department of the Army or the Department of Defense.

vae, with a 12 hr photoperiod. Adults were fed on guinea pigs 3 times weekly and maintained between blood meals on 10% sucrose solution. *Ae. taeniorhynchus* laid eggs on damp sphagnum moss and *Ae. aegypti* eggs were collected on moist paper toweling. Larvae were maintained in large rearing tubs (43 cm × 55 cm, 2000 larvae per tub in 7 liters dH_2O) and were fed a 1:1:1 mixture of liver powder, brewer's yeast and ground hog chow. In order to insure that larvae tested on a given day were all the same age, special egg hatching procedures were employed. *Ae. aegypti* eggs were soaked in dH_2O for 30 min and then force hatched for 30 min under vacuum in a common laboratory desiccator. The egg paper was then removed to prevent further hatch. *Ae. taeniorhynchus* eggs were allowed to hatch for 2 hrs in 0.3% saline, after which time all unhatched eggs were removed by pipette.

BIOASSAY PROCEDURE. Laboratory reared mosquitoes were bioassayed in 148 ml waxed paper cups in a total volume of 100 ml of dH_2O. In the case of *Ae. taeniorhynchus,* 0.3% salt water was used (Sea Salt, Natural Sales Co., Pittsburgh, PA). Larvae of desired age were selected at random and placed in groups of 20 (with 19 ml dH_2O) into 70 ml of dH_2O in the paper cups. One ml of food slurry (2.5 g 1:1:1 powder in 100 ml dH_2O) was added to each cup followed by the appropriate dilution of the BTI formulation in 10 ml dH_2O. The containers were then transferred to an incubator set at 27°C and 70% RH until mortality was recorded at 24 hrs and 48 hrs post-treatment. Thus, the larvae were exposed continuously to BTI. Five age groups were tested for both species: 6 hr, 24 hr, 48 hr, 72 hr, and 96 hr post-hatch. At 27°C, 6 hr and 24 hr old larvae were 1st stage, 48 hr were mostly 2nd stage, 72 hr were mostly 3rd stage and 96 hr old larvae were mostly 4th stage. Five groups of untreated controls (100 larvae) were also run during each test.

The BTI was supplied by Abbott Laboratories, Chicago, IL as an experimental dry powder formulation (lot #6406-125). (We have recently been informed by Dr. Terry Couch, Abbott Laboratories, that more active experimental formulations will soon be available.) The appropriate amount of powder was weighed and placed in a known volume of dH_2O in a Waring blender and mixed at maximum speed for 1 min. No wetting agent was necessary. The appropriate dilutions were then added to each cup of 20 larvae from a continuously agitated stock solution. Fresh aqueous solutions of BTI were prepared weekly and kept refrigerated after mixing.

Usually 8 or 9 doses were tested during each experiment, although up to 16 were used occasionally. Each dose was replicated 4 times during each test. The assay for a given age group was then repeated on either 2 or 3 separate occasions. Thus, a minimum of 240 larvae was tested at any given age group and dose.

Experiments were conducted so that at least 5 doses would give mortalities between 10% and 90%. Data for each age group were combined and probit analysis was performed on an IBM 370/168 computer using a Statistical Analysis System (SAS) program (Barr et al. 1976). Results were expressed as LD_1 through 99 with 95% confidence limits. Control mortality was consistently low (1% or less) and was not corrected for. In order to obtain some indication of additional mortality that followed the usual testing period of 48 hrs, two experiments were continued, one for 72 hr and one for 96 hr post-treatment.

Additional bioassays were conducted with 48 hr old larvae of both mosquito species using BTI material supplied by the Institut Pasteur (designated IPS 78). Four replicates (20 larvae each) for each dose were used for each of two separate experiments (160 larvae/dose). LD_{50}'s and LD_{90}'s calculated from tests run with Abbott's material were compared with those from the IPS 78 material in order to assess relative activity. The IPS 78 material contained 1000 international units per mg (IU/mg) so that a dose of 1 ppm or 1 mg/liter was equivalent to 1000 IU/liter.

RESULTS

As expected, younger larvae were more susceptible to BTI than the later stages (Table 1). In the case of *Ae. aegypti,* there appear to be 2 natural groupings. The LD_{50} for the 6 hr and 24 hr old larvae was approximately 0.1 ppm after 24 hr exposure while the LD_{50} for the 48 hr, 72 hr and 96 hr old larvae was about 0.4 ppm after 24 hrs. With *Ae. taeniorhynchus,* there was a steady progression from 0.03 ppm as the LD_{50} for 6 hr old larvae through 0.128, 0.578, 0.823 and finally 0.957 ppm for 96 hr old larvae after a 24 hr exposure.

Figures 1 and 2 graphically depict the effect of age of the larvae on their susceptibility to BTI. Again, LD_{50} and LD_{90} values are shown to be grouped for *Ae. aegypti* but evenly spaced for *Ae. taeniorhynchus.* It is also quite apparent from the figures, especially Fig. 1, that while early instar *Ae. taeniorhynchus* were more susceptible than *Ae. aegypti,* later instar *Ae. taeniorhynchus* were less susceptible than later instar *Ae. aegypti.*

When averaging data for all 5 age groups following a 24 hr exposure, the LD_{90} is roughly 2.5 times higher than the LD_{50} for both species. After a 48 hr exposure, the corresponding value is 3.0. (Compare Figs. 1 and 2, which are drawn to the same scale.)

Data from bioassays of 48 hr old larvae using the IPS 78 material is presented in Table 2. Comparing data from the Abbott material with that of the IPS 78 material, the relative activity varied from 0.332 to 0.590. On the average, the IPS 78 material is about 2.5 times as toxic as the Abbott material used in this test system.

In the two experiments in which mortality was recorded beyond the usual 48 hr, mortality continued to increase with exposure time. In 48 hr old larvae of *Ae. taeniorhynchus,* mortality was only 2.5% at the 0.10 ppm dose after 24 hr. It increased to 8.75% by 48 hr, to 15% by 72 hr and to 45% by 96 hr. In 72 hr old larvae, 0.50 ppm produced 16.25% mortality in 24 hr, 30% in 48 hr, and 66.25% in 72 hr.

Table 2. Susceptibility of 48 hr old larvae of *Aedes aegypti* and *Aedes taeniorhynchus* to *Bacillus thuringiensis israelensis* following continuous exposure for 24 hr and 48 hr: Comparison of Abbott's product to IPS 78 Material. (95% fiducial limits are in parentheses.)

	Aedes aegypti					
	LD_{50}			LD_{90}		
	IPS 78[1]	Abbott[1]	R.A.[2]	IPS 78	Abbott	R.A.
24 hr Exposure	0.166 (.157–.175)	0.458 (.424–.501)	0.362	0.307 (.284–.337)	0.926 (.754–1.397)	0.332
48 hr Exposure	0.153 (.134–.171)	0.364 (.329–.394)	0.420	0.284 (.246–.353)	0.849 (.705–1.183)	0.335
	Aedes taeniorhynchus					
	LD_{50}			LD_{90}		
	IPS 78	Abbott	R.A.	IPS 78	Abbott	R.A.
24 hr Exposure	0.256 (.237–.274)	0.578 (.359–.775)	0.443	0.635 (.574–.716)	1.776 (1.261–2.291)	0.358
48 hr Exposure	0.197 (.182–.212)	0.334 (.138–.528)	0.590	0.470 (.428–.525)	1.077 (.667–1.487)	0.436

[1] Expressed as ppm (w/v).

[2] R.A.=Relative Activity=$\frac{LD_x \text{ IPS } 78}{LD_x \text{ Abbott.}}$

Table 1. Susceptibility of *Aedes aegypti* and *Aedes taeniorhynchus* to *Bacillus thuringiensis israelensis* (Abbott Laboratories) as affected by larval age following continuous exposure for 24 hr and 48 hr (95% fiducial limits are in parentheses).

Aedes aegypti

Age of Larvae	6 hr		24 hr		48 hr		72 hr		96 hr	
# of Larvae Treated	240		240		320		320		240	
	LD_{50}[1]	LD_{90}[1]	LD_{50}	LD_{90}	LD_{50}	LD_{90}	LD_{50}	LD_{90}	LD_{50}	LD_{90}
Exposure Time										
24 hr	.099 (.090–.109)	.417 (.362–.495)	.122 (.104–.139)	.373 (.312–.480)	.458 (.424–.501)	.926 (.754–1.397)	.398 (.370–.428)	.738 (.649–.891)	.421 (.408–.434)	.868 (.824–.922)
48 hr	.063 (.056–.071)	.288 (.254–.332)	.077 (.069–.084)	.296 (.271–.328)	.364 (.329–.394)	.849 (.705–1.183)	.360 (.335–.384)	.687 (.610–.814)	.394 (.381–.406)	.808 (.770–.855)

Aedes taeniorhynchus

Age of Larvae	6 hr		24 hr		48 hr		72 hr		96 hr	
# of Larvae Treated	320		240		320		320		320	
	LD_{50}	LD_{90}	LD_{50}	LD_{90}	LD_{50}	LD_{90}	LD_{50}	LD_{90}	LD_{50}	LD_{90}
Exposure Time										
24 hr	.030 (.025–.035)	.067 (.054–.092)	.128 (.120–.137)	.293 (.266–.328)	.578 (.359–.775)	1.776 (1.261–2.291)	.832 (.655–.989)	2.098 (1.661–2.535)	.957 (.341–1.489)	2.148 (1.412–2.884)
48 hr	.022 (.021–.023)	.052 (.048–.057)	.065 (.028–.161)	.285 (.126–.444)	.334 (.138–.528)	1.077 (.667–1.487)	.520 (.268–.734)	1.456 (1.011–1.901)	.585 (.154–.906)	1.593 (1.011–2.175)

[1] LD_{50} and LD_{90} values expressed as ppm (w/v).

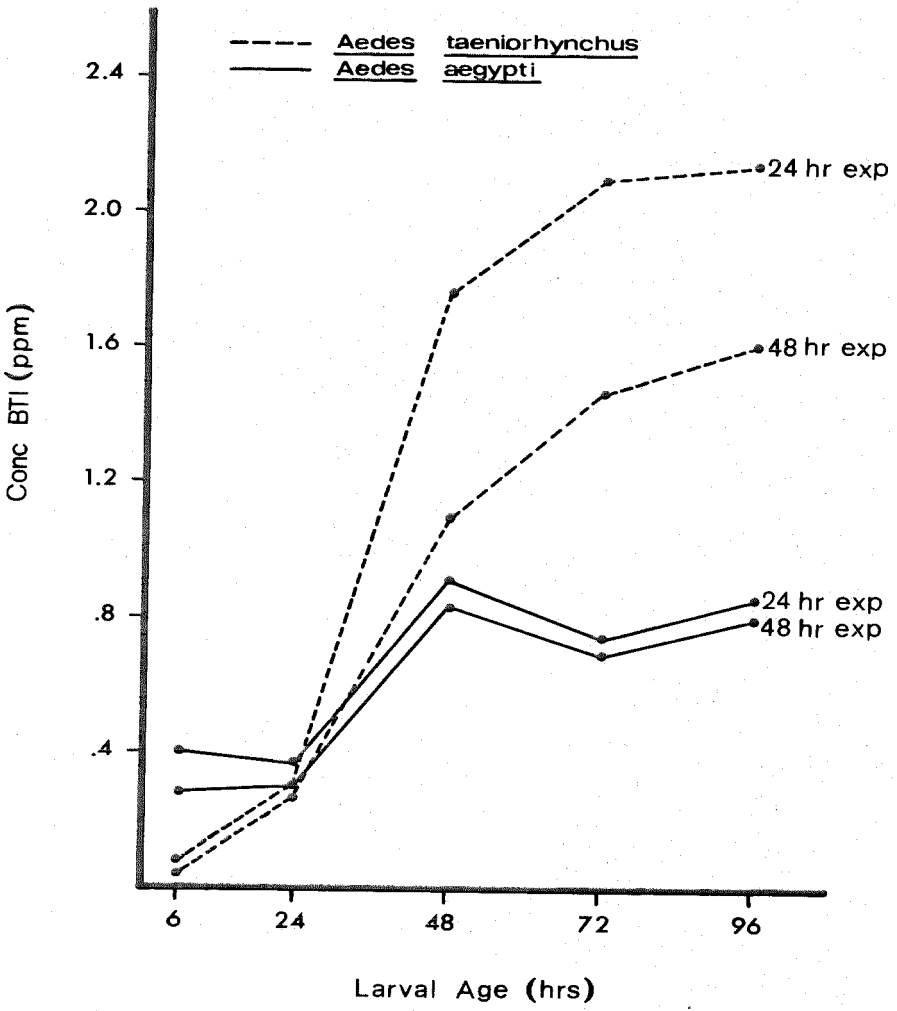

Figure 1. Comparison of LD_{90}'s of *Aedes taeniorhynchus* and *Aedes aegypti* at 5 larval ages following continuous exposure to *Bacillus thuringiensis israelensis* (Abbott Laboratories) for 24 hr and 48 hr.

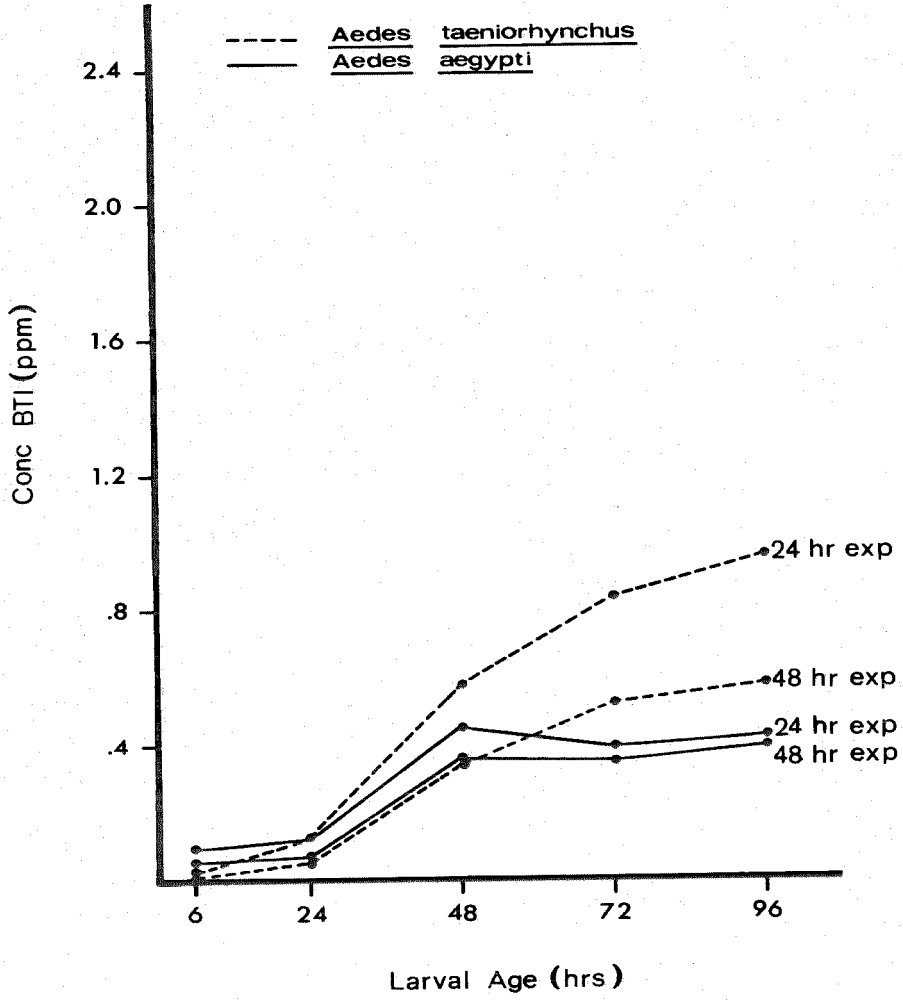

Figure 2. Comparison of LD_{50}'s of *Aedes taeniorhynchus* and *Aedes aegypti* at 5 larval ages following continuous exposure to *Bacillus thuringiensis israelensis* (Abbott Laboratories) for 24 hr and 48 hr.

DISCUSSION

Comparative susceptibility of target species may vary with the species of bacterial pathogen to which they are being exposed. De Barjac (1978b) found *Ae. aegypti* to be more susceptible to BTI than *Anopheles stephensi,* but both mosquito species were equally susceptible to *Bacillus sphaericus.* In the present study, *Ae. taeniorhynchus* was added to the list of susceptible species and was found to be less sensitive than *Ae. aegypti* in all larval age groups except the 6 hr one.

Earlier larval stages were more susceptible to BTI than the later ones for both mosquito species tested. This has been reported previously with pathogens and pesticides (Pimprikar et al. 1979, Maddox 1975, Canning 1970, de Barjac 1978b), although Goldberg and Margalit (1977) reported very little difference between 1st to 2nd and late 3rd to 4th instar *An. sergentii* and *Cx. pipiens* challenged with BTI. While some variability is exhibited in the data, as pointed out by Maddox (1975), this is to be expected because two living and, thus variable, entities are involved: the host and the pathogen. Working with *Ae. sierrensis,* Garcia and Desrochers (1979) reported that mortality at one concentration of BTI varied from 0 to 92%.

Comparison of susceptibilities of various mosquito species determined in different studies is difficult because of the number of methods employed in acquiring and reporting mortality data. Data reported as ppm of one formulation is not necessarily comparable to ppm of another. In general, spore counts are not a reliable index of potency (Dulmage 1971, Dulmage et al. 1971). The age of insect being tested is also important. In this study, 6 hr old *Ae. taeniorhynchus* were more susceptible than *Ae. aegypti,* whereas all other age groups were less susceptible. The LD_{50} and LD_{90} data have been reported in terms of ppm of dry formulation to demonstrate species and age differences in susceptibility, as well as in terms of relative activity, using standard material from the Institut Pasteur.

Preliminary observations indicate the need for studies beyond the usual 48 hr exposure time. In this system fairly large differences were noted. Hall et al. (1977) terminated their tests at the end of 7 days or at the onset of pupation; however, differences in mortality at 24 or 48 hr intervals were not reported. Goldberg and Margalit (1977) reported most activity occurring in less than 12 hrs. This would depend on how high a dose is used. De Barjac (1978c) reported an increase in mortality at one dose from 64% at 48 hr to 80% at 72 hr with 2nd stage larvae of *Ae. aegypti.*

One point to consider is that in most of these studies only mortality data is presented. While this is to be expected in preliminary work, it should be emphasized that other effects will be occurring, such as reduced adult longevity and fecundity in those individuals that survive the larval treatment with BTI. Longer term follow-up studies are needed to elucidate such phenomena.

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EDITORIAL NOTES

ABBREVIATIONS FOR NAMES OF GENERA

Mosquito News is beginning to follow the lead of *Mosquito Systematics* in adopting abbreviations for names of genera and subgenera as recommended by J. F. Reinert, 1975, *Mosquito Systematics* 7(2):105–110. The first time a generic or subgeneric name appears in an article it should be spelled out. Thereafter the appropriate abbreviation proposed by Reinert should be used. Recognition is given to Alan Stone who originated many of the abbreviations. The use of standardized abbreviations prevents confusion and reduces the amount of printing, especially in tables and lists. In *Mosquito News* subgeneric names are rarely repeated, so these abbreviations are not listed here. There follows Reinert's list of:

ABBREVIATIONS OF GENERA OF CULICIDAE

Aedeomyia	=*Ad*	*Malaya*	=*Ml*
Aedes	=*Ae*	*Mansonia*	=*Ma*
Anopheles	=*An*	*Maorigoeldia*	=*Mg*
Armigeres	=*Ar*	*Mimomyia*	=*Mi*
Bironella	=*Bi*	*Opifex*	=*Op*
Chagasia	=*Ch*	*Orthopodomyia*	=*Or*
Coquillettidia	=*Cq*	*Phoniomyia*	=*Ph*
Culex	=*Cx*	*Psorophora*	=*Ps*
Culiseta	=*Cs*	*Sabethes*	=*Sa*
Deinocerites	=*De*	*Topomyia*	=*To*
Eretmapodites	=*Er*	*Toxorhynchites*	=*Tx*
Ficalbia	=*Fi*	*Trichoprosopon*	=*Tr*
Galindomyia	=*Ga*	*Tripteroides*	=*Tp*
Haemagogus	=*Hg*	*Udaya*	=*Ud*
Heizmannia	=*Hz*	*Uranotaenia*	=*Ur*
Hodgesia	=*Ho*	*Wyeomyia*	=*Wy*
Limatus	=*Li*	*Zeugnomyia*	=*Ze*