*Journal of the American Mosquito Control Association,* 20(4):412–417, 2004


# EFFECTS OF SUBLETHAL CONCENTRATIONS OF VECTOBAC® ON BIOLOGICAL PARAMETERS OF *AEDES AEGYPTI*

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ABSTRACT. The effect of sublethal concentrations (30% lethal concentration [$LC_{30}$] = 0.41 ppm, $LC_{50}$ = 1.04 ppm, and $LC_{70}$ = 2.60 ppm) of VectoBac® 12 aqueous suspension (AS, *Bacillus thuringiensis* var. *israelensis* H-14, 600 ITU/mg) on life parameters of *Aedes aegypti* and its $F_1$ progeny (not exposed) was assessed in laboratory tests. Based on the data, it was clear that concentrations of 0.41 ppm of VectoBac significantly shortened the duration of the developmental cycle of the exposed mosquitoes, but not that of the $F_1$ (not exposed). Significant differences were found among the proportions of the age-specific survival between each toxic level, whereas the control did not differ from the treated individuals at the $LC_{50}$ and $LC_{70}$. The survival curves of the $F_1$ showed significant differences among the different treatments and with the control. A significant effect was found on the fecundity of adults. Age-specific fecundity was markedly lower for the $LC_{50}$ and $LC_{70}$ treatments compared to the $LC_{30}$ treatment and the control. In general, life parameters were affected inversely and significantly at higher concentrations of VectoBac, both in the exposed population of *Ae. aegypti* and in the $F_1$ (not exposed).

**KEY WORDS** *Bacillus thuringiensis* var. *israelensis,* VectoBac, *Aedes aegypti,* sublethal effects

## INTRODUCTION

Larvicidal agents, if administrated at high enough concentrations and rates, will yield complete or almost complete mortality in exposed populations. However, in practice, under diverse environmental conditions, it is not possible to achieve uniform coverage of the treated habitat, exposing all target organisms to uniform, lethal concentrations of a larvicide. It is a foregone conclusion that in nature some organisms will experience exposure to lethal or above-lethal concentrations, whereas others will be exposed to only sublethal doses. A number of chemical larvicides and mosquito control agents have been shown to manifest delayed effects (beyond the treated stage) at sublethal doses in the survivors. Effects of such delayed mortality include reduced survival of mature insects, reduction in the production of viable eggs, and reduction in fecundity and survival of $F_1$ individuals. Some of these types of effects have been documented, for example, for insect growth regulators (IGRs) in mosquitoes (Arias and Mulla 1975).

Studies on the delayed effects of *Bacillus thuringiensis* var. *israelensis* (*Bti*) on mosquitoes are scant. There are some indications that sublethal doses of *Bti* produce delayed effects beyond the stage treated. Hare and Nasci (1986) noted some delayed mortality in surviving larvae of *Aedes aegypti* (L.) exposed to a median lethal concentration ($LC_{50}$) of *Bti*. However, they did not detect any other noticeable negative effects on larvae surviving sublethal concentrations.

In other studies, Saleh and Wright (1989) and Saleh et al. (1987, 1990) studied the effects of *Bti* on the development and morphogenetic characteristics and reproductive potential of *Culex pipiens* L. Mulla and Singh (1991) examined in detail the delayed mortality, postemergence survival, and morphogenetic aberrations induced in surviving larvae, pupae, and adults of *Culex quinquefasciatus* Say after larvae were treated with sublethal concentrations ($LC_{25}$ and $LC_{80}$) of *Bacillus thuringiensis* H-14. Mulla et al. (1991) also studied these types of delayed effects with *Bacillus sphaericus* strain 2362 by using technical powder of flowable concentrate, whereas Lacey et al. (1987) reported on the delayed effects of the microbial agent strain 1593.

Some authors have indicated that in the laboratory, inefficient larviciding reduces larval competition among the survivors, and increases the density and the average body size of the resulting adult population (Agudelo-Silva and Spielman 1984). If this trend found in the laboratory occurs in the field, inefficient larviciding with *Bti* could produce a mosquito population with a higher vector potential than if the control measure had not been applied. However, if the survivors are adversely affected by the sublethal pesticide exposure, the vector potential of the adult population could be decreased.

The purpose of this study was to determine the effect of sublethal concentrations ($LC_{30}$, $LC_{50}$, and $LC_{70}$) of *Bti* when using VectoBac® aqueous suspension (AS) on survival, longevity, fecundity, and sex ratio of adults from surviving larvae and their $F_1$ progeny.

## MATERIALS AND METHODS

A commercial product of *Bti* (H-14), VectoBac® 12 AS (600 ITU/mg, Abbott Laboratories, North Chicago, IL), was utilized to determine the effects

Table 1. Mean developmental time in days (mean ± SE) and female : male (F:M) sex ratio of *Aedes aegypti* surviving from larvae exposed to sublethal concentrations of *Bacillus thuringiensis* var. *israelensis* (VectoBac® AS) and their $F_1$ progeny (not exposed).[1]

| | Exposed | | $F_1$ | |
|---|---|---|---|---|
| Concentration | Mean ± SE | F:M | Mean ± SE | F:M |
| $LC_{30}$ | 7.5 ± 1.19a | 1.00:1.04 | 18.5 ± 1.80a | 1.00:1.03 |
| $LC_{50}$ | 19.5 ± 1.84b | 1.00:1.00 | 23.5 ± 2.02a | 1.00:1.20 |
| $LC_{70}$ | 19.5 ± 1.84b | 1.00:1.40 | 24.0 ± 2.02a | 1.00:1.20 |
| Control | 17.0 ± 1.73b | 2.49:1.00 | 17.0 ± 1.73a | 2.49:1.00 |

[1] Values in the same column with the same letter did not differ significantly (F = 0.05).

of sublethal concentrations on biological parameters of *Ae. aegypti.* The sublethal concentrations considered in this study were based on the $LC_{30}$ (0.41 ppm), $LC_{50}$ (1.04 ppm), and $LC_{70}$ (2.60 ppm) obtained by Ponce et al. (2002) to determine the toxicity of the product in populations of *Ae. aegypti* from Monterrey, Nuevo Leon, Mexico.

A cohort of 1,500 eggs was used that originated from a colony of *Ae. aegypti* established in the insectary of the Medical Entomology Laboratory at the University of Nuevo Leon. This colony originally was obtained from flower vases in the city of Camp Saint El Roble in Monterrey, Nuevo Leon State, in northeastern Mexico. The eggs began to hatch after a period of 24 h and the larvae obtained were reared in 30 × 30-in. plastic trays. Ground dog food was used as larval food. Laboratory ambient conditions were 70% relative humidity and 28–29°C. The photoperiod was maintained at 12:12 light : dark. Larvae at the 2nd and 3rd instar were exposed to different sublethal concentrations ($LC_{30}$, $LC_{50}$, and $LC_{70}$) of *Bti* for 24 h. A total of 250 larvae per concentration were exposed in trays containing 2,000 ml of deionized water, with the same number of larvae without larvicide serving as the control. The surviving larvae were transferred to containers with clean water. Observations were carried out every 24 h to record the time of development. Emerging adults were counted and sexed to determine the sex ratio. The adult mosquitoes were placed in cages and fed on 10% sugar water. Mouse blood served as food for female mosquitoes. Survival, fecundity, and longevity of the females were recorded every 24 h until the last female died. All the biological parameters were determined for the surviving population after exposure to sublethal concentrations of larvicide and for their $F_1$ progeny (not exposed) as well. The data were analyzed according to standard procedures for life tables (Birch 1948).

Analysis of variance (ANOVA) and comparison of means by Tukey's test ($P = 0.05$) were utilized to compare total and mean daily fecundity during the development cycle with the total number of females obtained at the different concentrations of larvicide. The survival curves were compared by means of the log-rank test (Mendez et al. 1984) for the survivors of the exposed population and for their $F_1$ progeny as well.

## RESULTS AND DISCUSSION

### Development cycle

An analysis of the mean developmental time (7.5 days) of *Ae. aegypti* surviving from larvae exposed to sublethal concentrations of the larvicide indicates a significant difference ($P = 0.05$) at the $LC_{30}$ with respect to the rest of the treatments and the control. In other words, an exposure to low concentrations of *Bti* significantly shortened the duration of the development cycle. However, upon con-

Table 2. Population parameters of *Aedes aegypti* surviving from larvae exposed to sublethal concentrations of *Bacillus thuringiensis* var. *israelensis* (VectoBac® AS) and their $F_1$ progeny (not exposed).

| | Exposed | | | | $F_1$ | | |
|---|---|---|---|---|---|---|---|
| Parameter | Control | $LC_{30}$ | $LC_{50}$ | $LC_{70}$ | $LC_{30}$ | $LC_{50}$ | $LC_{70}$ |
| Net reproductive rate ($R_0$) | 0.558 | 3.562 | 20.450 | 12.630 | 5.140 | 9.300 | 6.940 |
| Gross reproductive rate (GRR) | 239.100 | 364.530 | 352.400 | 281.100 | 469.000 | 427.200 | 430.000 |
| Finite growth rate ($\lambda$) | 0.989 | 1.030 | 1.100 | 1.060 | 1.030 | 1.050 | 1.040 |
| Time of cohort ($T_c$) | 56.070 | 47.850 | 44.770 | 48.070 | 61.410 | 51.480 | 47.950 |
| Growth capacity ($r_c$) | −0.010 | −0.026 | 0.067 | 0.052 | 0.027 | 0.043 | 0.040 |
| Intrinsic growth rate ($r_m$) | −0.010 | −0.030 | 0.099 | 0.063 | 0.030 | 0.051 | 0.046 |
| Mean generation time ($T_G$) | 5.684 | 42.530 | 30.420 | 39.790 | 54.450 | 43.610 | 47.950 |
| Instantaneous birth rate ($b$) | 0.201 | 0.205 | 0.134 | 0.139 | 0.157 | 0.167 | 0.177 |
| Instantaneous mortality rate ($d$) | 0.211 | 0.175 | 0.035 | 0.076 | 0.127 | 0.116 | 0.131 |
| Doubling time ($T_2$) | −67.702 | 23.100 | 6.980 | 10.870 | 23.040 | 13.550 | 14.940 |

Table 3. Periods of preoviposition, oviposition, postoviposition, and longevity in days of female *Aedes aegypti* emerged from larvae surviving different sublethal concentrations of VectoBac® AS and their $F_1$ progeny (not exposed).

| Period (days) | Exposed | | | $F_1$ | | | Control |
|---|---|---|---|---|---|---|---|
| | $LC_{30}$ | $LC_{50}$ | $LC_{70}$ | $LC_{30}$ | $LC_{50}$ | $LC_{70}$ | |
| Preoviposition | 5 | 6 | 7 | 5 | 6 | 12 | 10 |
| Oviposition | 97 | 106 | 89 | 100 | 108 | 95 | 46 |
| Postoviposition | 8 | 3 | 2 | 6 | 10 | 10 | 5 |
| Longevity | 112 | 118 | 100 | 114 | 120 | 120 | 64 |

tinuing the evaluation in the $F_1$ progeny resulting from the exposed population, no apparent significant difference was found in this parameter among the treatments and control (Table 1).

## Sex ratio

The results (Table 1) indicate a higher proportion of males in the majority of the treatments, with the exception of adults arising from larvae exposed to the $LC_{50}$, in which 1:1 sex ratio was found. Meanwhile, the female : male ratio was 2.49:1 in the control.

The largest difference occurred in the population exposed to the $LC_{70}$, in which the female : male sex ratio was 1:1.4. Based on the results obtained here, it can be concluded that sex ratio did not differ among individuals exposed to different sublethal concentrations of *Bti*. However, when comparing the results for these sublethal concentrations with that of the control, it is evident that this larvicide did have an influence on the sex ratio. A similar case occurred in a study by Juarez Equia (1990), who obtained a larger proportion of females in the $F_1$ progeny of individuals exposed to the $LC_{10}$ and $LC_{50}$ of temephos. In our study, the proportion of females was reduced with *Bti* treatments, indicating that the treated populations are at a disadvantage because there would be a decrease in the reproductive population.

## Growth parameters

The results obtained for growth are presented in Table 2, and are based on survival and fecundity tables and standard procedures for life tables (Birch 1948).

The results show a decrease in gross reproductive rate (GRR) with increasing concentrations of *Bti*. Both the exposed individuals and their $F_1$ progeny showed greater values of GRR at the $LC_{30}$ (364.5:409, exposed : $F_1$), when compared with the

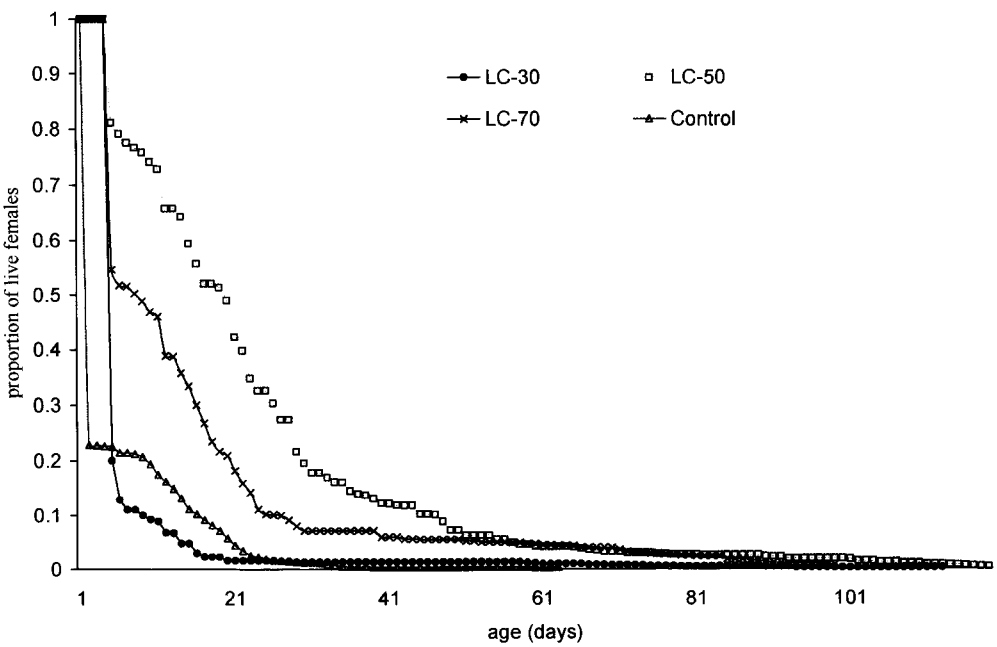


Fig. 1. Surivorship curves for female *Aedes aegypti* emerged from larvae surviving different sublethal concentrations of VectoBac® AS.

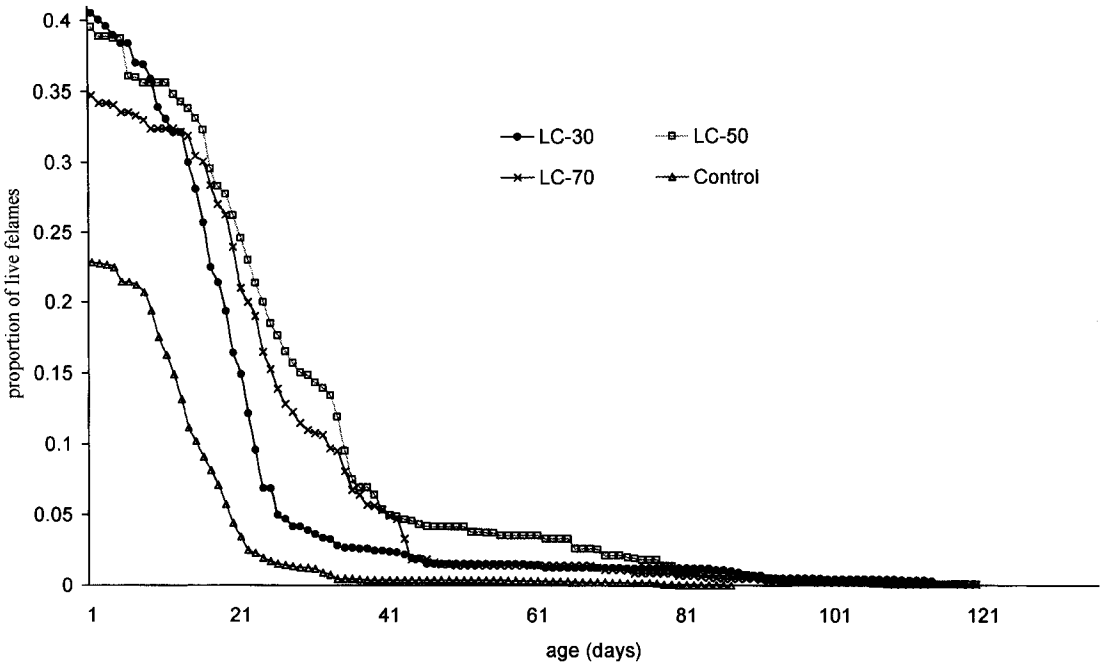


Fig. 2. Survivorship curves of female *Aedes aegypti* ($F_1$ progeny) from parents exposed to sublethal concentrations of VectoBac® AS.

$LC_{70}$ (281.1:430, exposed:$F_1$). In all cases, GRR values of the $F_1$ progeny for the 3 concentrations tested were greater than those of the exposed population and the control (239.1). The reduction in GRR with increase in *Bti* concentration demonstrated in this case that at higher larvicidal concentrations, females show a lower reproductive potential, which was reflected in a decline in total fecundity (total daughters born per mother) exposed to concentrations higher than the $LC_{50}$.

With regard to the generation time ($T_G$), the results indicate a shorter duration of 30.4 and 43.6 days with $LC_{50}$ concentration for the exposed and $F_1$ groups, respectively, which led to a daily population increase by a factor of 1.03 and 1.03, respectively. There was a longer generation time of 42.5 and 54.4 days for the exposed and $F_1$ progeny, respectively, at the $LC_{30}$ and with a daily increase in population by a factor of 1.10 and 1.05, respectively. The generation time observed with all the sublethal concentrations was significantly longer than in the control.

The intrinsic growth rate ($r_m$) was higher for the $LC_{50}$ in the exposed individuals and the $F_1$ progeny, with values of 0.09 and 0.05, respectively, and was lower for the $LC_{30}$, being 0.03 in both exposed and $F_1$ individuals. In this case, lower values were obtained compared to those reported by Lansdowne and Hacker (1975), who determined an intrinsic growth rate ($r_m$) of 5 lines of *Ae. aegypti* under controlled conditions for temperature and relative humidity of 27 ± 1°C and 70%. The lines utilized were Carrizal, Ocala, Newala-Bamboo, Newala-House, and Houston. The values of the intrinsic period of growth for the exposed individuals and their $F_1$ progeny, respectively, for the above lines in order were 0.4057 and 0.3973, 0.4251 and 0.4204, 0.4628 and 0.4719, 0.4243 and 0.4383, and 0.4192 and 0.4274.

The net reproductive rate ($R_0$) was higher with

Table 4. Mean daily fecundity (±SE) of female *Aedes aegypti* emerged from larvae surviving to different sublethal concentrations of VectoBac® AS and their $F_1$ progeny (not exposed).

| Concentration | Exposed | $F_1$ |
|---|---|---|
| $LC_{30}$ | 3.37 ± 0.427 | 4.22 ± 0.406 |
| $LC_{50}$ | 3.09 ± 0.267 | 3.71 ± 0.442 |
| $LC_{70}$ | 2.92 ± 0.282 | 3.73 ± 0.510 |
| Control | 3.32 ± 0.734 | 3.62 ± 0.791 |

Table 5. Mean values (±SE) for total daily fecundity of female *Aedes aegypti* emerged from larvae surviving different sublethal concentrations of VectoBac® AS and their $F_1$ progeny (not exposed).

| Concentration | Exposed | $F_1$ |
|---|---|---|
| $LC_{30}$ | 39.79 ± 6.05 | 89.47 ± 9.27 |
| $LC_{50}$ | 176.50 ± 21.38 | 119.28 ± 14.65 |
| $LC_{70}$ | 216.36 ± 25.54 | 57.64 ± 7.90 |
| Control | 26.34 ± 4.35 | 28.74 ± 4.64 |

the $LC_{50}$ for the exposed individuals and their $F_1$ progeny as well (Table 2), with respective values of 20.45 and 9.301. These values were lower with the $LC_{30}$ at 3.58 and 5.14 for the exposed and $F_1$ progeny, respectively, and much lower in the control at 0.055. We obtained lower values than those reported by Lansdowne and Hacker (1975), who determined the net reproductive rate ($R_0$) of the 5 lines of *Ae. aegypti* mentioned above, obtaining values of 110.94 and 93.05, 141.13 and 123.13, 166.92 and 174.60, 152.98 and 154.38, and 235.78 and 228.02, respectively.

## Oviposition

The results for the oviposition and pre- and post-oviposition periods in females both arising from exposed larvae and their $F_1$ progeny, including the control, are shown in Table 3. The preoviposition period in both the exposed individuals and their $F_1$ progeny increased with an increase in *Bti* concentration, whereas the time of oviposition increased at the $LC_{50}$ and decreased at a higher concentration ($LC_{70}$). A similar case was seen with the time of postoviposition, which increased with an increase in *Bti* concentration for the exposed individuals, but not for the $F_1$ progeny.

In this case, the effect of VectoBac AS can be seen on the aforementioned parameters, because the increase in the preoviposition time and decrease in the oviposition time would diminish as a consequence the number of generations. In addition, as a result of another effect evidently produced by the larvicide, the longevity of females arising from exposed larvae decreases with an increase in concentration of the larvicidal agent, which is reflected in a lower number of days for oviposition.

## Survival

As mentioned in the Materials and Methods, the survival curves (Figs. 1 and 2) of the individuals exposed to different sublethal concentrations, as well as the $F_1$ progeny, were compared by means of the log-rank method. A significant difference ($P < 0.05$) was found in the exposed individuals in survival by specific age among all the treated groups, whereas the control did not differ from the treated individuals at the $LC_{50}$ and $LC_{70}$. With respect to the $F_1$ progeny, the survival curves showed significant differences among the different treatments, and among these and the control.

According to Slobodkin (1964), the types of survival curves that were seen with *Ae. aegypti* exposed to the different concentrations described above are of type III for individuals exposed to the $LC_{50}$ and $LC_{70}$, whereas those for the control and those exposed to the $LC_{30}$ are of type IV. This signifies that the mortality rate at the beginning of the cycle was greater for the latter 2 groups. With respect to the $F_1$ progeny, the types of survival curves that resulted were also of type III and IV, although in this case the control was the only group that showed a type IV curve.

## Fecundity

Mean daily fecundity (Table 4) did not differ significantly (ANOVA, $P < 0.05$) among females derived from the exposed and unexposed (control) larvae. Similarly, their $F_1$ progeny also did not show any significant differences in fecundity.

With respect to total daily fecundity, the individuals treated with the $LC_{50}$ or $LC_{70}$ differed significantly ($P < 0.05$) from those exposed to the $LC_{30}$ or control. In the $F_1$ progeny, total daily fecundity of the control and $LC_{70}$-treated individuals differed significantly compared to those treated with the $LC_{30}$ or $LC_{50}$. The mean values for total daily fecundity of both the exposed individuals and their $F_1$ progeny are shown in Table 5.

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