# EFFECTS OF SUBLETHAL EXPOSURE TO *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* ON LARVAL DEVELOPMENT AND ADULT SIZE IN *AEDES AEGYPTI*

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ABSTRACT. The effects of exposure to sublethal concentrations of *Bacillus thuringiensis* var. *israelensis* (Serotype H-14) on second instar *Aedes aegypti* larvae were investigated. A test system was developed in which adverse effects would be detected as increased duration of larval development and decreased adult body size. No evidence of negative effects on survivors could be detected when sufficient *B.t.i.* dosages were applied to kill approximately half of the larvae in the treatment groups. However, when larval density was not controlled, and competition for food decreased as a result of larval mortality in the *B.t.i.*-treated groups, adult wing length was greater in the *B.t.i.* survivors than in the untreated controls. In addition, a residual mortality was noted in larvae that had been exposed to *B.t.i.* for 24 hr and then removed to habitats without *B.t.i.*

## INTRODUCTION

*Bacillus thuringiensis* var. *israelensis* (Serotype H-14; *B.t.i.*) is a safe, effective biological control agent (Davidson and Sweeney 1983, Legner and Sjogren 1984), but one of its drawbacks is that its efficiency is affected by numerous environmental conditions (Garcia and Des Rochers 1979). This, coupled with its rapid biodegradation (Garcia et al. 1980), could result in *B.t.i.* producing less than 100% mosquito mortality in many habitats.

In the laboratory, inefficient larviciding has been shown to reduce larval competition among the survivors, and increase the density and the average body size of the resulting adult population (Agudelo-Silva and Spielman 1984). If this occurs in the field, inefficient larviciding with *B.t.i.* 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.

Since larval development rate is slowed and adult body size is reduced by reduced food availability (McCombs 1980[1], Livdahl 1984, Istock et al. 1975, Wada 1965, Patrican and DeFoliart 1985) and *B.t.i.* disrupts the midgut epithelia (Davidson and Sweeney 1983), it is possible that sublethal exposure to *B.t.i.* may affect nutrient assimilation and result in longer larval development periods and smaller adults. Thus, sublethal exposure to *B.t.i.* could control the population by exposing the larvae to predation for a longer period of time, and by reducing adult size, which has been related to reduced survival and blood-feeding success in the field (McCombs 1980,[1] Haramis 1983, Nasci 1986).

The purpose of this study was to determine if exposure to sublethal amounts of *B.t.i.* has an effect on the larvae surviving to the adult stage. The parameters that were investigated are duration of larval development and adult body size.

## MATERIALS AND METHODS

*Aedes aegypti* (Linn.) (Rockefeller strain) was the mosquito used in this study. Larvae were grown at 27 ± 1° C, 16 hr light/8 hr dark, in an 11.5 cm diam. × 5 cm deep glass bowl containing 200 ml deionized water. Fifty first-instar larvae were placed in each bowl. Larvae were fed a suspension of liver powder (Cat. #900396, ICN Nutritional Biochemicals). The liver powder was passed through a 100 mesh sieve prior to suspension in water to insure uniformity of particle size. Pupae were placed in plastic cages (11.4 cm diam. × 7.6 deep) and held at 27 ± 1° C until adult emergence.

The influence of nutrient availability on larval survival, rate of immature development, and adult body size in *Ae. aegypti* was verified by varying the amount of food supplied to larvae grown in the otherwise constant conditions described above. The high diet level provided 0.4 mg liver powder/larva while the low diet level provided 0.05 mg/larva. Each diet regime was replicated 5 times. Larval survival and development rates were determined by collecting pupae daily from each bowl.

Adult body size was determined by measuring the wing length (distance from the axial incision to the apical margin, excluding the fringe of scales) of each adult individual. Wing length was chosen as an indicator of size because it is directly proportional to dry body weight (Christophers 1960, McCombs 1980[1])

[1] McCombs, S. D. 1980. Effect of differential nutrition of larvae on adult fitness of *Aedes triseriatus*. Unpublished Master's Thesis, University of Notre Dame, Notre Dame, IN.

and does not change over the life of the adult (Christophers 1960). Measurements were made by removing the right wing of each mosquito, placing it on a glass slide, and measuring the length with a dissecting microscope equipped with an ocular micrometer.

Teknar®, a water-dispersible concentrate of *B.t.i.* containing 1,500 *Ae. aegypti* Toxic Units/mg (Zoecon Corp., 12200 Denton Dr., Dallas, TX) was the source of toxin used in this study. Sublethal dosages of *B.t.i.* were determined by placing fifty 72 hr-old *Ae. aegypti* larvae in glass rearing bowls containing 200 ml of Teknar in various concentrations (10 ppm to 0.1 ppm). No food was provided to the larvae during the treatment period. After 24 hr, surviving larvae were removed to fresh water and counted. Mortality rates were determined and the $LC_{50}$ calculated according to Reed and Muench (1938). Further study showed that although this $LC_{50}$ produced 50% mortality at the end of 24 hr, all surviving larvae died within 72 hr after removal from the Teknar solution. Therefore, another $LC_{50}$ was determined based on a 24 hr exposure to concentrations (0.1 ppm to 0.001 ppm) of Teknar that resulted in 50% of the larvae surviving to pupation.

The effect of sublethal *B.t.i.* exposure on larval development and adult size was determined by placing 72 hr old *Aedes aegypti* larvae into bowls (50 larvae/200 ml deionized water), adding Teknar to produce a final concentration of 0.001 ppm, and holding the bowls in a controlled-temperature incubator at 19 ± 1 °C for 24 hr. The surviving larvae in each bowl were then removed with a pipette from the *B.t.i.* solutions, placed in a clean bowl (50 larvae/200ml deionized water) and started on the standardized high level larval diet. Bowls of 50 larvae, treated identically except for the addition of Teknar, served as controls. Each treatment was replicated 5 times. Pupation rates and wing length measurements were determined as described above. In order to control for differences in larval development, larval survival and adult size due to different levels of competition for food (Wada 1965), larval density was kept the same in each bowl (50 ± 5 larvae) by pooling larvae as they pupated or died.

A second experiment, identical except for the maintenance of constant larval density in the bowls and the use of several Teknar concentrations (0.1, 0.01, 0.001 ppm) was conducted to determine if larvae surviving *B.t.i.* benefited from the reduction in competition for food. In this experiment, each bowl initially contained 50 larvae, but dead larvae and pupating mosquitoes were not replaced.

All statistical tests were performed using methods described in Sokal and Rohlf (1969) and with the Statistical Package for the Social Sciences (Nie et al. 1975).

## RESULTS

The experimental manipulations of larval diet demonstrated that food availability significantly affected larval development and larval survival. The high diet produced a shorter larval development period (average = 13 days for high diet, 31 days for low diet) and a higher larval survival rate (99.2% for high diet, 92.8% for low diet). The high level diet produced adults with significantly larger wing lengths (high diet males 2.03 ± 0.07 mm, low diet males 1.86 ± 0.09 mm, $p \leq 0.001$, ANOVA; high diet females 2.75 ± 0.15 mm, low diet females 2.28 ± 0.21 mm, $p \leq 0.001$ ANOVA).

The first $LC_{50}$ calculated for Teknar, based on the number of survivors at the end of a 24 hr exposure, was 0.212 ppm. This $LC_{50}$ could not be used in subsequent experiments since none of the larvae that survived 24 hr exposure to the *B.t.i.* at this concentration survived to pupation. The second $LC_{50}$, based on a 24 hr exposure to a concentration of Teknar that would allow 50% of the larvae to survive to pupatation, was between 0.01 and 0.001 ppm Teknar. This $LC_{50}$ was difficult to standardize, probably due to variations in temperature and handling techniques.

In the first experiment, where a density of 50 ± 5 larvae per bowl was maintained, no differences were detected in larval development rates or adult wing lengths between the treated and untreated groups (Table 1).

In the second experiment, where competition for food in the treatment bowls decreased as mortality reduced larval density, *B.t.i.* had little effect on the duration of larval development (Table 2). Adult female wing lengths were significantly larger in the treated population than in the control population. Male wing lengths did not display a consistent trend (Table 2).

## DISCUSSION

Consistent with previous observations, dietary levels influenced larval survival rates, larval development and adult body size. This indicates that adverse effects on nutrient assimilation, due to exposure of larvae to sublethal concentrations of *B.t.i.*, would be detectable as increased larval development time or decreased adult body size in the experimental system that was used.

The attempt to determine an $LC_{50}$ for Teknar revealed the existence of two different

Table 1. Comparison of larval development, larval survival and adult size among *B.t.i.*-treated and untreated control groups when larval density was held constant.

| | Treatment | |
|---|---|---|
| | Control | 0.001 ppm Teknar |
| Day of: | | |
| First pupation | 10 | 10 |
| 50% pupation | 11 | 11 |
| Last pupation | 16 | 16 |
| % larval survival | 84.5 | 58.7* |
| Male wing length (mm) | 1.94 ± 0.09 | 1.93 ± 0.08 |
| N | 86 | 53 |
| Female wing length (mm) | 2.47 ± 0.17 | 2.49 ± 0.14 |
| N | 55 | 46 |

* Larval survival in the treated group is based on the number of larvae surviving a 24 hr exposure to the *B.t.i.* concentration shown.

$LC_{50}$s, depending on the definition of $LC_{50}$ that is used. Normally $LC_{50}$s are calculated using percent mortality at the end of a 24 hr exposure to a toxin. The observation of 100% mortality within 72 hr after larvae were removed from the *B.t.i.* indicates that *B.t.i.* has residual effects extending beyond 24 hr. This indicates that 24 hr mortality rates probably underestimate the effect of *B.t.i.* and that smaller amounts of *B.t.i.* may be used in field applications.

Although *B.t.i.* causes mortality even after larvae are removed from the *B.t.i.*, larvae that survive exposure to *B.t.i.* do not appear to suffer any ill effects in the form of reduced body size or extended larval development. This was demonstrated in the first experiment in which larval density and competition for nutrients were kept constant. These results suggest that *B.t.i.* has an all or nothing effect. Either the mosquitoes die from exposure or survive unaffected.

The second experiment, in which competition for food was reduced by larval mortality in *B.t.i.*-treated bowls, verified the observation by Agudelo-Silva and Spielman (1984). Inefficient larviciding can increase the body size of the surviving adults. In this case, *B.t.i.*-treated survivors developed as rapidly as untreated larvae and produced adults that were significantly larger than untreated adults.

## ACKNOWLEDGMENTS

The authors thank the Calcasieu Parish Mosquito Control Program for supplying the Teknar used in this study.

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Table 2. Comparison of larval development, larval survival and adult size among *B.t.i.*-treated and untreated control groups when larval density is not regulated.

| | | Teknar concentration (ppm) | | |
|---|---|---|---|---|
| | Control | 0.1 | 0.01 | 0.001 |
| Day of: | | | | |
| First pupation | 9 | 9 | 9 | 9 |
| 50% pupation | 11 | 11 | 11 | 11 |
| Last pupation | 20 | 13 | 16 | 16 |
| % larval survival[1] | 93.2 | 41.6 | 79.2 | 99.6 |
| Male wing length (mm)[2] | 1.99 ± 0.08[b] | 1.97 ± 0.11[ab] | 1.93 ± 0.12[a] | 1.98 ± 0.10[b] |
| N | 100 | 53 | 62 | 91 |
| Female wing length (mm)[2] | 2.70 ± 0.17[a] | 2.75 ± 0.17[b] | 2.76 ± 0.14[b] | 2.76 ± 0.12[b] |
| N | 119 | 48 | 94 | 104 |

[1] Larval survival in the Teknar treated groups is based on the number of larvae surviving a 24 hr exposure to the concentration of Teknar shown.

[2] Values with different letters are significantly different ($p \leq 0.05$, Student, Newman, Keuls).

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