# DELAYED MORTALITY AND MORPHOGENETIC ANOMALIES INDUCED BY THE MICROBIAL CONTROL AGENT *BACILLUS THURINGIENSIS* SER. (H-14) IN *CULEX QUINQUEFASCIATUS*

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ABSTRACT. Sublethal concentrations of *Bacillus thuringiensis* ser. H-14 were applied to early 4th-instar larvae of *Culex quinquefasciatus* to assess mortality and morphogenetic aberrations in larvae, pupae and adults. At the 24 h $LC_{10}$, $LC_{25}$, $LC_{50}$ and $LC_{80}$, additional mortality occurred in surviving larvae beyond a 24 h exposure period. The cumulative mortalities increased daily and the overall mortality of larvae up to 7 days posttreatment were 12, 73, 82 and 96% at the indicated concentrations, respectively. Delayed mortality also occurred in the pupal and adult stages. Morphogenetic aberrations were noted in dead larvae and pupae but were rare in the adults. These aberrations are categorized and described. There was little or no delayed effect on the survivorship or fecundity of adults, but in all the treatments the number of emerging males was higher than females. The sex ratio in check adults was 1:1.

## INTRODUCTION

In recent years many studies have been carried out on the activity and efficacy of microbial control agents against mosquito larvae. Among the microbial larvicides, *Bacillus thuringiensis* serotype H-14 ( *B.t.* H-14) has attracted considerable attention as a possible substitute for some chemical control agents against mosquito larvae. This entomopathogen has been found to show a good level of activity and efficacy against numerous species of mosquitoes (Lacey and Singer 1982, Lacey et al. 1984, Mulla and Darwazeh 1984, Mulla et al. 1986, Perich et al. 1988). Additionally, it has a good margin of safety to aquatic nontarget organisms prevailing in both mosquito and blackfly control programs (Lacey and Mulla 1991).

In standard bioassay tests in the laboratory and field evaluation of *B.t.* H-14, the mortality readings are taken 24 h after start of the exposure period. No attempt has been made, except in a limited number of studies, to assess delayed mortality in cohorts or members of stages resulting from the surviving larvae subjected to sublethal concentrations of *B.t.* H-14. In recent studies Saleh and Wright (1989) and Saleh et al. (1987, 1990) studied its effects on the development and morphogenetic characteristics and reproductive potential of *Culex pipiens* Linn. Mulla et al. (1991) studied delayed mortality and morphogenetic aberrations induced in surviving larvae, pupae and adults of *Cx. quinquefasciatus* Say when larvae were treated with sublethal concentrations of the entomopathogen *Bacillus sphaericus* Neide strain 2362 while Lacey et al. (1987) studied delayed effects of strain 1593 of this microbial agent.

From the above studies it seems that both microbial control agents produce delayed mortality and that *B. sphaericus* strain 2362 induces morphogenetic aberrations at high frequencies in the surviving larvae, pupae and adults (Mulla et al. 1991). The present studies were implemented to assess delayed mortality and types and magnitude of morphogenetic aberrations in larvae, pupae and adults of *Cx. quinquefasciatus* fed *B.t.* H-14. Additionally, the impact of sublethal concentrations of *B.t.* H-14 on sex ratios and longevity of adults resulting from the cohorts surviving sublethal treatments was assessed.

## MATERIALS AND METHODS

A primary powder of *Bacillus thuringiensis* H-14 (ABG-6164 provided by Abbott Laboratories, North Chicago, IL) was used. Methods for the evaluation in the laboratory were similar to those described elsewhere (Mulla et al. 1991). In brief, fresh stock suspensions (1%) of bacterial powder were prepared in distilled water, and serial dilutions of 0.1, 0.01, 0.001 and 0.0001% were prepared in distilled water as needed. The latter 2 suspensions were used in bioassay tests. Four concentrations within the activity range were tested. In each test 60 larvae were exposed (20 larvae in 100 ml per cup) at each concentration and the test repeated 3 times on different occasions. Mean percent mortality was plotted against concentration and the log concentration regression line was fitted as described by Mulla et al. (1991). Tests were performed on a laboratory strain of *Cx. quinquefasciatus*. Values of $LC_{10}$, $LC_{25}$, $LC_{50}$ and $LC_{80}$ were obtained from the established dosage-response line and chosen for treating early 4th-instar larvae.

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Tests for determining delayed mortality and effects were performed at a temperature of 27 ± 1°C with 14L:10D photoperiod. The initial larval mortality was recorded after 24 h. The surviving larvae were transferred to clean fresh distilled water in cups, larval food was given soon after transfer and once again during the 7 day experiment. The larvae were reared to pupae and adults, and delayed mortality was recorded every day until all the survivors either died or emerged as adults. The dead specimens were scrutinized daily for morphogenetic aberrations.

In determining the bioactivity and delayed mortality in the larvae up to 7 days posttreatment, 4 concentrations were employed using 20 larvae in each of 15 cups for each concentration or check (total of 300 larvae). The test was repeated on 5 different occasions (except as stated in the tables) thus using a total of 1,500 larvae each time. Appropriate controls were maintained by using specimens of the same age.

Data on sex ratio, longevity, and fecundity of adults were obtained in other tests using a concentration of 0.015 mg/liter ($LC_{50}$). To assess the adult fecundity and longevity, 10–15 females and 5 male mosquitoes from the pool of surviving adults in the treated and checks were placed in each of 4 screened cages maintained in a room at 27 ± 1°C and photoperiod of 14L:10D. The females were blood fed on chicks 2–3 days after emergence and then again every 3–4 days before each gonotropic cycle. Oviposition cups containing tap water were placed in the cages overnight. After every blood meal, all the cages were checked daily for egg rafts and for adult mortality. Eggs deposited were allowed to hatch and the extent of egg raft hatch was ascertained.

## RESULTS AND DISCUSSION

In the first experiment *B.t.* H-14 (ABG-6164) powder was used at 4 concentrations ($LC_{10}$, $LC_{25}$, $LC_{50}$ and $LC_{80}$) obtained from the log dosage-response line established for ABG-6164 to give approximately 10, 25, 50 and 80% initial larval mortality. The concentrations corresponding to these LC values were 0.075, 0.01, 0.015 and 0.02 mg/liter, respectively.

The initial mortality after 24 h exposure period was 6, 29, 50 and 84% at 0.075, 0.01, 0.015 and 0.02 mg/liter, respectively.

Additional mortality after the initial 24 h was recorded at each concentration. The cumulative or overall mortality (based on the initial number) of larvae after one week was 12, 73, 82 and 96% for the 0.075, 0.01, 0.015 and 0.02 mg/liter concentrations, respectively, as compared with 0.9, 2.6, 1.9 and 1.4% in checks. Thus, additional delayed mortality can occur at sublethal concentrations. The percent mortality as expected decreased with time because the number of survivors decreased in succeeding cohorts. Delayed lethal action of *B.t.* H-14 was also observed by Saleh and Wright (1989) using 0.05–0.6 ppm aqueous suspension of *B.t.* H-14 (ABG-6193) in *Aedes epacticus* Dyar and Knab.

Treatment of early 4th-instar larvae with sublethal concentrations (0.015 mg/liter, $LC_{50}$) of *B.t.* H-14 produced a number of morphogenetic aberrations in dead larvae. The aberrations were categorized into 5 types as follows, and the results are given in Table 1.

A. The most common aberration (in 15% of the larvae) was an elongated neck region with or without the presence of a transparent anterodorsal thoracic bulbous projection. This was also the most common aberration in dead larvae treated with *B. sphaericus* 2362 (Mulla et al. 1991).

B. Larvae shortened and crumpled.

C. Larvae normal in shape but jet black.

D. Larvae partially black.

E. Prepupae—where all the external characteristics were those of larvae, but the pupae were formed and contained within the larval exoskeleton. The respiratory and digestive tracts were not clearly visible because the abdomen was opaque.

Occurrence of morphogenetic aberrations was very rare in the check population. As noted in the previous experiment, a small proportion (17.5%) of larvae survived the $LC_{50}$ treatment which developed into pupae (17%) and adult stages (16%) as based on the initial numbers of larvae. Additional mortality in pupae and adults resulting from the surviving larvae was not high at all concentrations tested (Table 2). As the concentration increased, mortality in larvae increased, resulting in fewer pupae and adults. As shown in Table 2, there was little mortality

Table 1. Occurrence of morphogenetic aberrations induced by a sublethal concentration of *Bacillus thuringiensis* H-14 (ABG-6164) at 0.015 mg/liter ($LC_{50}$) in dead *Culex quinquefasciatus* larvae (early 4th instar).

| Aberration[a] categories | No. with aberrations | % larvae with aberrations[b] |
|---|---|---|
| A | 230[c] | 15 |
| B | 73 | 5 |
| C | 77 | 5 |
| D | 31 | 2 |
| E | 47 | 3 |

[a] Described in text.

[b] Percentage based on the initial number of larvae used (1,500).

[c] 39 (2.5%) with anterodorsal transparent bulbous projection.

Table 2. Delayed mortality in *Culex quinquefasciatus* pupae and adults resulting from larvae surviving sublethal concentrations of *Bacillus thuringiensis* H-14 (ABG-6164).

| Concentration mg/liter | % pupal[a] mortality (no.) | Mean % and (no.) of dead adults as[a] | |
|---|---|---|---|
| | | Partially emerged | Completely emerged |
| 0.01 ($LC_{25}$) | 2.5 (38) | 0.6 (10) | 1.0 (16) |
| 0.0 check | 0.5 (8) | 0.0 (0) | 0.5 (7) |
| 0.015 ($LC_{50}$) | 1.7 (27) | 0.4 (6) | 0.6 (10) |
| 0.0 check | 0.5 (7) | 0.0 (0) | 0.6 (9) |
| 0.02 ($LC_{80}$) | 0.4 (8) | 0.0 (0) | 0.3 (5) |
| 0.0 check | 0.4 (6) | 0.0 (0) | 0.0 (0) |

[a] Percentage calculated on the basis of the starting number of larvae (1,500 total) per concentration or check.

(calculated on the basis of the starting larvae) at the 2 high concentrations. This is in agreement with the findings of Saleh and Wright (1989). However, Mulla et al. (1991), recorded high mortality in pupae and adults of *Cx. quinquefasciatus* that survived sublethal concentrations of *B. sphaericus* 2362. The dead pupae in the *B.t.* H-14 treatments, though small in number, had morphogenetic anomalies such as being larviform, darkened, elephantoid (with enlarged anterior end and protruding trunk) and partially molted. These morphogenetic aberrations were quite similar to those observed in *B. sphaericus* 2362 treatments reported by Mulla et al. (1991). However, no morphogenetic aberrations were found in adults, a phenomenon that is in contrast to *B. sphaericus* effects (Mulla et al. 1991).

Adult males and females developing from the survivors of larvae treated with the sublethal concentration of 0.015 mg/liter ($LC_{50}$) showed similar longevity as those in check populations. Male mosquitoes were shorter lived in both the treated and check groups (Table 3). By gonotropic cycle 4, 80% of the males had died while only 24% of the females had died. There was a decrease in oviposition rates with aging. The hatching rate of egg rafts in the treated and checks was the same in each gonotropic cycle. This is in agreement with the findings of Mulla et al. (1991) in treatments with *B. sphaericus* 2362.

Table 4 shows that survivors of larval treatments with $LC_{50}$ produced more males than females in the treatment groups as compared with the checks. There was an overall ratio of 1.4 males/females in the treatment and a ratio of 0.96 males/females in the checks. It seems that immatures destined to be females are more sensitive to the treatments, thus reflecting the effects of the toxin on the larvae before pupation, resulting in ratios skewed toward the males in the treatments.

In recent investigations on the effects of a sublethal concentration of *B.t.* H-14 on the reproductive potential of *Cx. pipiens*, Saleh et al. (1990) found more females than males emerged in the treatment groups as compared with checks in the first generation. They also recorded that egg production and hatchability of eggs was reduced in the first generation but not in the second generation. In our studies, we assessed only sex ratio and fecundity of the emerging adults resulting from larvae surviving the sublethal treatment. Therefore, the results

Table 3. Posttreatment effects of *Bacillus thuringiensis* H-14 (ABG-6164) on the longevity and fecundity of *Culex quinquefasciatus* adults resulting from larval treatments at 0.015 mg/liter ($LC_{50}$).[a]

| Gonotropic cycle | Mean no. males surviving[b] per cage | Mean no. females surviving[c] per cage | Mean no. egg rafts per cage | Egg raft hatch | |
|---|---|---|---|---|---|
| | | | | Mean no. per cage | % |
| 1 Treated | 5.0 | 12.5 | 10.8 | 9.0 | 83 |
| Check | 5.0 | 12.5 | 9.8 | 9.0 | 92 |
| 2 Treated | 3.5 | 11.0 | 9.0 | 8.0 | 89 |
| Check | 3.8 | 11.7 | 8.5 | 7.5 | 88 |
| 3 Treated | 1.8 | 9.7 | 7.5 | 6.8 | 91 |
| Check | 1.5 | 9.0 | 7.0 | 6.5 | 93 |
| 4 Treated | 1.0 | 9.5 | 8.5 | 7.5 | 88 |
| Check | 1.0 | 9.0 | 7.0 | 6.0 | 86 |
| 5 Treated | 0.3 | 7.3 | 6.8 | 5.5 | 81 |
| Check | 0.3 | 6.3 | 5.1 | 4.5 | 88 |
| 6 Treated | 0.0 | 5.3 | 3.5 | 3.0 | 86 |
| Check | 0.0 | 4.6 | 2.0 | 2.0 | 100 |

[a] Test repeated 4 times, using 1,200 larvae per treatment and check.
[b] Of the surviving males, 5 males were placed in each of 4 cages.
[c] Of the surviving females, 10 females were placed in each of two cages and 15 females in each of the other 2 cages, thus yielding a mean of 12.5 females/cage at the start.

Table 4. Effect of *Bacillus thuringiensis* H-14 (ABG-6164) on the sex ratio of adult *Culex quinquefasciatus* resulting from larvae surviving treatment (0.015 mg/liter) for 24 h.

| Test treatment[a] | Mean no./test (300 larvae)[b] | | Sex ratio (♂/♀) |
|---|---|---|---|
| | Males | Females | |
| 1 Treated | 27 | 19 | 1.4 |
| Check | 125 | 150 | 0.8 |
| 2 Treated | 23 | 15 | 1.5 |
| Check | 130 | 135 | 0.9 |
| 3 Treated | 26 | 16 | 1.6 |
| Check | 90 | 88 | 1.0 |
| 4 Treated | 21 | 12 | 1.7 |
| Check | 120 | 122 | 1.0 |
| 5 Treated | 32 | 30 | 1.0 |
| Check | 135 | 132 | 1.0 |
| Total treated | 129 | 92 | 1.4 |
| Total checks | 600 | 627 | 0.96 |

[a] 300 larvae used per test.

[b] Number of survivors from 300 larvae in treated and check each.

of the 2 studies are not comparable. It is, however, interesting to note that fecundity of the first generation adults raised from adults of the treated generation was lower than the checks (Saleh et al. 1990). Such delayed effects of treatments either on the contemporary generation or the succeeding generation(s) could have implications in terms of controlling mosquitoes with entomopathogens. All evaluation techniques assess efficacy 24 or 48 h posttreatment not accounting for the additional delayed mortality in the succeeding cohorts. The overall final reduction would be greater than the reduction assessed at the prevailing exposure periods. However, further studies are warranted to confirm such delayed effects beyond the treated contemporary generation cohorts.

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