# DELAYED MORTALITY AND MORPHOGENETIC ANOMALIES INDUCED IN *CULEX QUINQUEFASCIATUS* BY THE MICROBIAL CONTROL AGENT *BACILLUS SPHAERICUS*

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ABSTRACT. Two preparations of *Bacillus sphaericus* 2362 were studied for their biological activity, delayed mortality and the induction of morphogenetic aberrations in larvae, pupae and adults of *Culex quinquefasciatus*. Longevity and fecundity of adult mosquitoes were also assessed. A dosage response line for *B. sphaericus* was established against 4th-instar larvae and sublethal concentrations (48 h $LC_{50}$ and lower) were used against these larvae. Sublethal concentrations of *B. sphaericus* induced delayed mortality in larvae, pupae and adults. The magnitude of mortality increased in succeeding cohorts and developmental stages resulting from the surviving larvae. Only 10 and 25% overall emergence of viable adults occurred in the sublethal treatments ($LC_{25}$) of 2 *B. sphaericus* preparations. The range of successful adult emergence was over 94% in the controls. A wide range of external morphogenetic aberrations in dead larvae, pupae and adults were noted. These aberrations and gross morphological features were quite similar to those reported for certain insect growth regulators. Sublethal concentrations had no marked effect on longevity of adults, egg deposition and hatch.

## INTRODUCTION

In the past decade, research on the isolation, identification and development of entomopathogenic organisms for mosquito control has received due attention. Among these pathogens, *Bacillus sphaericus* Neide, a spore-forming bacterium has received much attention for possible development as a mosquito larvicide. Although this agent has not been registered for operational use in the United States, it is commercially available for operational mosquito control programs in several countries abroad.

*Bacillus sphaericus* has shown excellent biological activity against several genera of mosquitoes, but it is more specific and highly active against *Culex* mosquitoes (Davidson et al. 1984, Lacey and Singer 1982, Lacey et al. 1984; Mulla et al. 1984, 1986). Procedures for testing this agent against mosquito larvae in the laboratory utilize standard bioassay techniques in exposing larvae to suspensions of pathogen preparations in a volume of water in disposable cups or nondisposable glass or plastic containers. Since *B. sphaericus* is relatively slow acting, mortality readings are taken 48 h after start of exposure to various concentrations within the activity range of the preparations or formulations (Lacey and Singer 1982, Mulla 1986, Mulla et al. 1986). This exposure period (48 h) has been standardized and used by many researchers.

In our laboratory and field studies, we have noted that mosquito larvae surviving sublethal treatment with this agent and subsequent stages resulting from the survivors suffer additional mortality (beyond 48 h). This additional mortality was noted in the larval stage (treated) and the resulting pupae and adults which developed from the surviving larvae treated at sublethal concentrations. The additional or delayed mortality could further increase the overall activity range of *B. sphaericus* preparations. Studies were implemented to assess delayed posttreatment mortality of surviving larvae (treated at sublethal concentrations), and the resulting pupae and adults. To further elucidate the delayed effects, survivorship and fecundity of the female adults resulting from the surviving larvae were also assessed. A proportion of the surviving larvae (past 48 h mortality), resulting pupae and adults had morphogenetic anomalies. These aberrations were characterized and quantified in the present studies.

## MATERIALS AND METHODS

Two preparations of *B. sphaericus* strain 2362 were used in these studies. A primary powder, ABG-6184 technical powder (Abbott Laboratories, North Chicago, IL), had a spore count of $1 \times 10^{11}$. The other preparation, BSP-1, a flowable concentrate (FC) formulation (Biochem Products, Mountchanin, DE), had a spore count of $2 \times 10^{7}$. Both these formulations have been extensively studied in the laboratory and field (Mulla 1986).

To evaluate and quantify initial as well as delayed effects of these 2 preparations, stock suspensions (1% weight/volume basis) were prepared in distilled water. Serial dilutions (0.1, 0.01, 0.001 and 0.0001%) were made in distilled water. New stock and diluted suspensions were prepared fresh for each experiment. To obtain

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the desired concentrations, aliquots from the 0.0001% dilution were added to 100 ml water in cups containing mosquito larvae. After the exposure period of 48 h, survivors were removed; and delayed mortality, fecundity and morphogenetic aberrations in the individuals dying were assessed upon death.

For testing purposes, larvae from a laboratory colony of *Culex quinquefasciatus* Say were used. In each test 20 4th-instar larvae were placed in 140 ml disposable waxed paper cups (Sweetheart Ice Cream and Food Cups DS304, Fort Howard Cup Corp., Green Bay, WI), each containing 100 ml distilled water. The cups were replicated in each test anywhere from 75 to 125 (in 3–5 separate tests) cups for each concentration. During the preliminary tests to determine the activity of the 2 preparations, where mortality was merely assessed 48 h after exposure, no larval food was given the larvae. However, in tests where delayed mortality, morphogenetic effects and adult fecundity were studied, larval food (ground up lab chow and brewer's yeast 3:1) was provided initially as well as once more during the 7–8 day life span of the treated and check larvae.

To determine the bioactivity of the 2 preparations, they were tested against early 4th-instar larvae, using 4 concentrations (from 0.0025 to 0.01 mg/liter). Three cups containing 20 larvae each were used per concentration and check, each test repeated on 3 different occasions. Thus, a total of 180 larvae were used per concentration and check. The mean percent mortality (48 h) was plotted against concentration on log probit paper and a straight line fitted through the points. The $LC_{50}$ and $LC_{25}$ as well as lower LCs used in subsequent studies were read off the dosage response lines established.

In determining the delayed mortality in the larvae (up to 7 days posttreatment) and morphogenetic effects, the 48 h $LC_{50}$, $LC_{25}$ and even lower concentrations were employed, using 20 early 4th-instar larvae in each of 25 cups for each concentration or check. The test was repeated on 3 or 5 different occasions, thus employing 1,500–2,500 larvae for each concentration and check. To obtain adequate numbers of adult survivors for assessment of morphogenetic effects and mortality in the pupae at the $LC_{25}$ or lower concentrations, a large number of replicates were utilized. To determine the delayed mortality and morphogenetic aberrations, the surviving larvae were transferred after 48 h to cups containing untreated distilled water. These larvae were provided food twice and held in room with a temperature of 27 ± 1°C and photoperiod of 12L:10D with 1 h of dim light at dawn and dusk periods. The overall mortality was determined by the amount of successful adult emergence on the basis of the starting number of larvae. Incompletely emerged adults were counted as dead and so were emerged drowned adults.

To determine the longevity and fecundity of adult females resulting from surviving larvae treated at sublethal concentrations ($LC_{25}$), the surviving larvae were transferred to fresh water, provided food and allowed to pupate. About 1,500 larvae were used in each of the treated and untreated regimens. Pupae were removed daily from each cup, combined and placed in a screened wooden cage (30.5 × 30.5 × 22.8 cm) in a room with 27 ± 1°C and 12L:10D photoperiod and 1 h dim lights at dusk and dawn.

For characterizing and quantifying morphogenetic anomalies, dead larvae, pupae and adults were removed daily and preserved in 75% ethanol. The dead larvae, pupae or incompletely eclosed adults were counted and inspected under a stereoscopic microscope. Proportion (%) of the occurrence of a given type of the morphogenetic aberration was calculated for each stage on the basis of the surviving larvae, pupae and adults.

To assess the fecundity and longevity of the adults, 20 female and 10 male mosquitoes from the pool of the emerged adults were placed in each of 3 replicated screened cage maintained at 27 ± 1°C and photoperiod of 12L:10D photoperiod and 1 h of dim lights at dusk and dawn. The adults were provided with 5% sucrose solution. The females were blood-fed 3 days after emergence and then again every 3 to 4 days before each gonotropic cycle. The females were allowed to lay eggs in cups containing tap water. All egg rafts laid during a gonotropic cycle were left in the cup and hatching of egg rafts was assessed 2–3 days after they were laid.

Dead males and females were removed, and the number of surviving males and females was recorded in each cage. In this process, differential survivorship of males and females in the treated and check population was ascertained.

## RESULTS AND DISCUSSION

In the first experiment, ABG-6184 was used at $LC_{50}$ (0.004 mg/liter) and $LC_{25}$ (0.002 mg/liter), while BSP-1 (FC) was used at $LC_{25}$ (0.004 mg/liter) and $LC_{10}$ (0.003 mg/liter). The initial mortality (48 h) was in agreement with the established values for these preparations (Mulla 1986).

Noticeable additional mortality beyond the 48 h exposure period occurred even though the larvae were removed to untreated water. This additional mortality was 21, 11, 7, 5 and 1.3% on days 3, 4, 5, 6 and 7, respectively, at the $LC_{50}$

(0.004 mg/liter) level of ABG-6184 (Table 1). Similar additional delayed mortality occurred at the $LC_{25}$ (0.002 mg/liter) level of this product. The overall mortality of larvae during the 1 wk period was 96% for the $LC_{50}$ and 72% for the $LC_{25}$. Overall mortalities in the checks were negligible (1–3%). Lacey et al. (1987) also obtained significant delayed mortality when 2nd-instar larvae of *Cx. quinquefasciatus* were treated with a lyophilized product (RB-80) of *B. sphaericus* strain 1593.

Similar trends of increased mortality were noted for the BSP-1 formulation (Table 1). At the $LC_{25}$, the overall mortality was 64%; and at the $LC_{10}$, the overall mortality was 31%. Thus, with both preparations, additional delayed mortality was realized at sublethal concentrations. This picture of delayed mortality with sublethal concentrations of *B. sphaericus* is indeed a plus for this entomopathogen. On successive reduction of the survivors due to mortality, the percent mortality (based on the initial number of larvae) as expected decreased with time. If mortalities were based on the number of survivors, then the percent mortality calculations will be greater on successive days.

Delayed mortality in succeeding cohorts or stages resulting from surviving (48 h exposure) 4th-instar larvae treated in the previous experiment (Table 1) with the primary powder ABG-6184 at the $LC_{25}$ (0.002 mg/liter) and BSP-1 at the $LC_{25}$ (0.004 mg/liter) was ascertained. Of the few surviving larvae (28% of the treated population) in ABG-6184 treatment, further mortality (18%) occurred in prepupae, pupae and partially or completely emerged adults (Table 2). Considering the overall mortality of 72% (from Table 1) plus 18% in succeeding stages (Table 2) brings the total mortality to 90% for the 48 h $LC_{25}$ treatment. When larvae were treated with the sublethal concentration ($LC_{25}$), only 10% successfully emerged as adults based on the initial number of larvae. Most of the mortality after the larval stage occurred in pupae (Table 2). Also, somewhat reduced delayed mortality occurred in the sublethal treatment with BSP-1, most of the delayed mortality beyond the larval stage also occurring in pupae. Overall, only 25% successfully emerged as adults. Emergence in the checks was normal.

The dead pupae had various morphogenetic anomalies such as being larviform, darkened pupae and elephantoid (enlarged and swollen cephalic region). These morphogenetic aberrations are similar to those induced by insect juvenile hormones, their analogs and mimics (Arias and Mulla 1975, Awad and Mulla 1984). All morphogenetic anomalies were noted in dead larvae, pupae and adults.

Treatment of early 4th-instar larvae with sublethal concentrations of *B. sphaericus* ($LC_{25}$) produced a variety of morphogenetic aberrations in the succeeding stages resulting from the surviving larvae. The aberrations noted were numerous with gradations. Since all of the morphogenetic aberrations cannot be described here, only the more common and notable aberrations are described and quantified. The major types of aberrations were:

Larvae:

A. The most noticeable anomaly was the formation of a transparent anterodorsal thoracic bulbous projection and elongated neck region (Fig. 1A). The elongated neck feature also occurs in larvae dying from other causes, but the bulbous projection has not been noted before.
B. Abnormal shaped larvae, shortened lengthwise and crumpled (Fig. 1B).
C. Larvae possessing normal shape but were

Table 1. Initial and delayed activity of *Bacillus sphaericus* 2362 primary powder (ABG-6184) and BSP-1 liquid formulation against *Culex quinquefasciatus* larvae (early 4th instar) in the laboratory.

| Formulation | Concentration mg/liter | Mean (%) mortality in larvae during periods (days) posttreatment | | | | | | Overall % mortality |
|---|---|---|---|---|---|---|---|---|
| | | 2 | 3 | 4 | 5 | 6 | 7 | |
| ABG 6184[a] primary powder | 0.004 ($LC_{50}$) | 51.0 | 21.0 | 11.0 | 7.0 | 5.0 | 1.3 | 96 |
| | 0.0 | 1.0 | 0.0 | 0.7 | 0.3 | 0.0 | 0.0 | 2 |
| | 0.002 ($LC_{25}$) | 26.0 | 20.0 | 15.0 | 6.0 | 3.6 | 1.0 | 72 |
| | 0.0 | 1.0 | 0.0 | 1.0 | 0.3 | 0.3 | 0.0 | 3 |
| BSP-1[b] FC | 0.004 ($LC_{25}$) | 24.0 | 20.0 | 11.0 | 5.0 | 3.0 | 1.0 | 64 |
| | 0.0 | 0.3 | 1.0 | 0.0 | 0.0 | 0.7 | 0.0 | 2 |
| | 0.003($LC_{10}$) | 9.3 | 7.3 | 6.0 | 4.6 | 3.0 | 0.4 | 31 |
| | 0.0 | 0.0 | 0.0 | 0.3 | 0.7 | 0.0 | 0.0 | 1 |

[a] 20 larvae per cup and 25 cups per treatment and the test repeated 5 times, thus using 2,500 larvae/concentration and each check.

[b] 20 larvae per cup and 25 cups per treatment of this formulation, the test repeated 3 times, thus using 1,500 larvae per concentration and each check.

Table 2. Delayed mortality induced in pupae and adults resulting from surviving larvae following treatment of early 4th-instar larvae of *Culex quinquefasciatus* with *Bacillus sphaericus* 2362 (ABG-6184) and BSP-1 (FC) at the $LC_{25}$ (0.002 mg/liter and 0.004 mg/liter, respectively).

| Exp. no. | No. dead larvae[a] | No. pupated | % pupated[b] | No. dead pupae | No. dead adults as: Partially emerged | No. dead adults as: Completely emerged | No. adults surviving | Mean % overall emergence[c] |
|---|---|---|---|---|---|---|---|---|
| | | | *ABG-6184 (0.002 mg/liter)* | | | | | |
| 1 | 348 | 152 | 30.0 | 63 | 6 | 23 | 61 | 12 |
| 2 | 358 | 142 | 28.4 | 57 | 5 | 28 | 52 | 10 |
| 3 | 363 | 137 | 27.4 | 62 | 3 | 20 | 52 | 10 |
| 4 | 389 | 111 | 22.2 | 73 | 7 | 5 | 26 | 5 |
| 5 | 330 | 170 | 34.0 | 78 | 10 | 27 | 50 | 10 |
| Total | 1,788 | 712 | 28.4 | 333 | 29 | 103 | 241 | 10 |
| Check (mean for all experiments) | — | — | — | — | — | — | — | 94 |
| | | | *BSP-1 (0.004 mg/liter)* | | | | | |
| 1 | 311 | 189 | 38.0 | 50 | 3 | 8 | 128 | 26 |
| 2 | 338 | 162 | 32.4 | 36 | 4 | 12 | 110 | 22 |
| 3 | 322 | 178 | 35.6 | 42 | 1 | 5 | 130 | 26 |
| Total | 971 | 529 | 35.5 | 128 | 8 | 25 | 368 | 25 |
| Check (mean for all experiments) | — | — | — | — | — | — | — | 95 |

[a] 500 larvae used per experiment.
[b] On the basis of the starting number of larvae.
[c] On the basis of the starting number of larvae (500) per experiment.

jet black in color. Larvae dying from other causes also show this feature.

Pupae:

D. Freshly ecdysed pupae from larval skin, remaining light or pale; dying before hardening and melanization of the cuticle without or with larval exuviae (Fig. 1, C and D).
E. Pupae elongate, rod-like and larviform similar in length to 4th-instar larvae in general shape.
F. Black pupal form and extended.
G. Partially molted pupa with the larval head capsule exoskeleton still attached to the anterior portion of the pupal thorax with or without larval exuvium (Fig. 1E).

Adults:

H. Adults in which the anterior part of the body was eclosed, but the whole abdomen still remained within the pupal skin. Mouthparts and legs were seemingly curled and glued to the body.
I. Dead adults completely eclosed with crumpled wings.
J. Adults completely eclosed, except that the tarsi were still within the pupal skin, wings twisted (Fig. 1F).

In all the dead specimens observed, only one possessed a form intermediate between that of the larva and pupa. This specimen had a larval head capsule, and the thorax was still bearing setae. The respiratory tracheae were not clearly visible. The opaque abdomen lacked a siphon tube, saddle and setal tufts, and the caudal end had typical pupal paddles.

The distribution and proportion of morphogenetic aberrations were ascertained according to the above types and categories. Both formulations induced a fairly high level of morphogenetic aberrations (Table 3). The percentage of specimens with aberrations in the larval stage was based on the initial numbers of larvae. An overall 23 and 18% of the larvae had noticeable aberrations in ABG-6184 and BSP-1 treatments, respectively. About 49 and 46% of the dead larvae in the 2 treatments had no visible anomalies. Approximately 46 and 23% of the pupae had visible anomalies in the 2 treatments, respectively. Only about 1% of the dead pupae had no visible anomalies.

In the 2 treatments, about 23 and 8.2% of the dead adults exhibited visible and identifiable external anomalies. Only 13% of the dead adults (ABG-6184 treatment) showed no visible anomalies. Aberrations described above were rare in the check populations, because very few if any (less than 3%) died.

The distribution of the various types of anomalies in dead pupae is presented in Table 4. The percentage of pupae with each of the identified

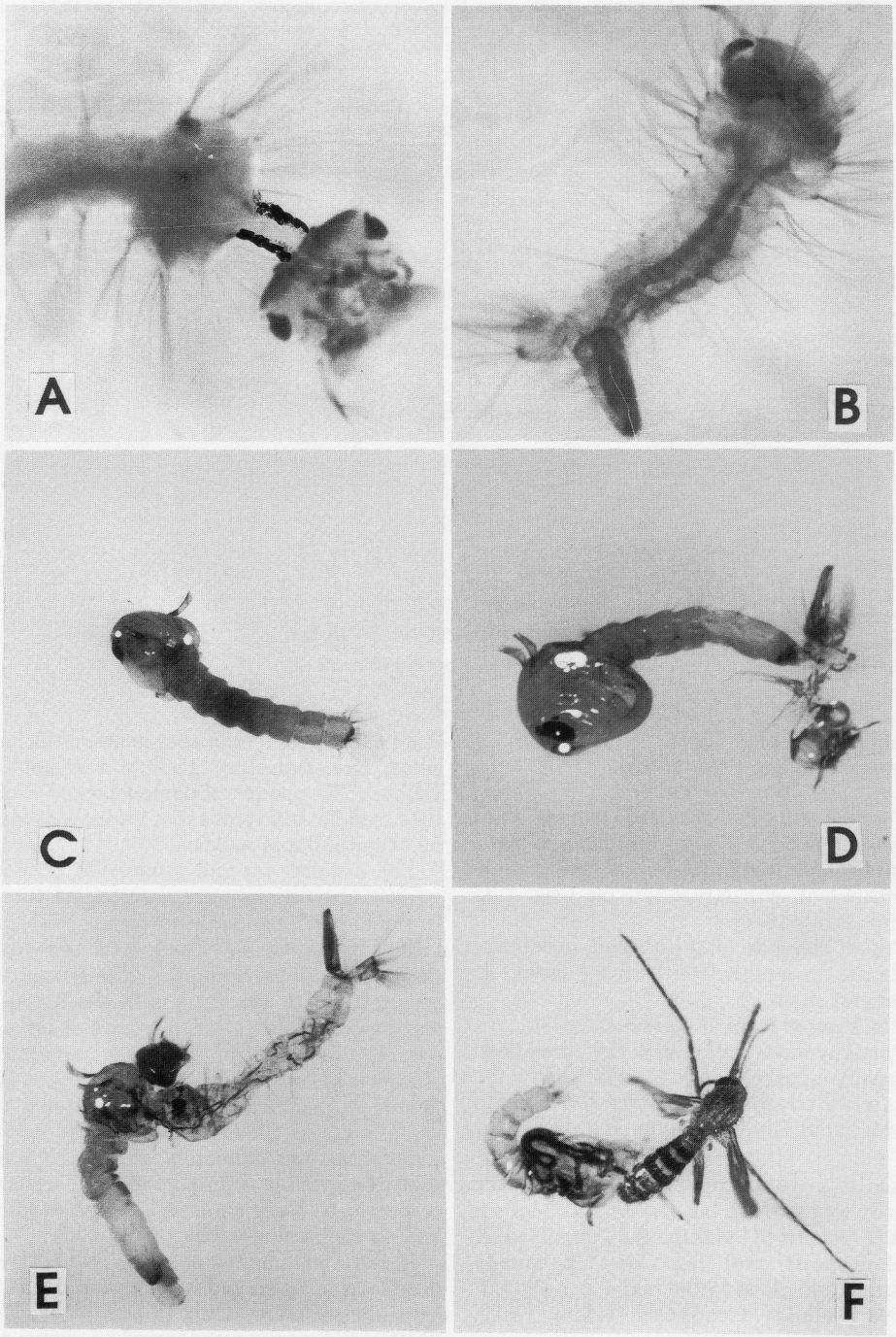


Fig. 1. A. Elongated neck region in larvae dead from treatment with *Bacillus sphaericus*; B. Shortened and shrunk larva dead in *B. sphaericus* treatment; C. Pupa of *Culex quinquefasciatus* developing from surviving larvae treated with sublethal concentration of *B. sphaericus*. The freshly ecdysed albino pupa was not completely melanized or hardened, and died soon after molting; D. Pupa with attached larval exuvium at the caudal end and pupa not completely melanized or hardened; E. Partly molted pupa with the larval head capsule exoskeleton attached to the anterior portion of the pupal thorax; F. Adult completely emerged but still attached to pupal exuvium by legs. The wings in these adults are twisted and they are unable to fly, thus dying on water surface.

Table 3. Occurrence of morphogenetic aberrations induced by sublethal concentration of *Bacillus sphaericus* (ABG-6184 at 0.002 mg/liter and BSP-1 at 0.004 mg/liter) in dead *Culex quinquefasciatus*.

| Aberration category[a] | No. with categorized aberrations | | % with aberrations based on total in each stage | |
|---|---|---|---|---|
| | ABG-6184 | BSP-1 | ABG-6184 | BSP-1 |
| | | *Larvae*[b] | | |
| A | 123 | 42 | 5.0 | 3.0 |
| B | 244 | 106 | 10.0 | 7.0 |
| C | 199 | 125 | 8.0 | 5.0 |
| Without aberrations (dead) | 1,222 | 698 | 49.0 | 46.5 |
| | | *Pupae*[c] | | |
| D | 68[d] | 33[e] | 9.5 | 6.2 |
| E | 72 | 32 | 10.0 | 6.0 |
| F | 168 | 48 | 24.0 | 9.0 |
| G | 17[f] | 10 | 2.4 | 2.0 |
| Without aberrations (dead) | 8 | 5 | 1.1 | 1.0 |
| | | Adults[g] | | |
| H | 22 | 8 | 6.0 | 2.0 |
| I | 55 | 23 | 15.0 | 6.0 |
| J | 7 | 2 | 2.0 | 0.5 |
| Without aberrations (dead) | 48 | 0 | 13.0 | 0.0 |

[a] Categories are described in text.
[b] Percentage based on the initial number of larvae used (2,500 for ABG-6184 and 1,500 for BSP-1). The percentages will be greater if based on the number of surviving larvae.
[c] Percentage based on the total no. pupated.
[d] 32 with larval exuviae attached.
[e] 15 with larval exuviae attached.
[f] Eight with larval exuviae attached.
[g] Percentage based on the total no. of adults emerged.

Table 4. Occurrence of some morphogenetic aberrations in dead pupae induced by sublethal concentration of *Bacillus sphaericus* (ABG-6184 at 0.002 mg/liter and BSP-1 at 0.004 mg/liter) used against larvae.

| Category of aberration | No. pupae died[a] | | % dead[b] | |
|---|---|---|---|---|
| | ABG-6184 | BSP-1 | ABG-6184 | BSP-1 |
| Albino | 68 | 33 | 9.5 | 6.2 |
| Larviform | 72 | 32 | 10.0 | 6.0 |
| Black | 63 | 20 | 8.8 | 3.8 |
| Elephantoid | 35 | 11 | 5.0 | 2.0 |
| Enlarged distended | 60 | 11 | 8.4 | 2.0 |
| Partially molted | 17 | 10 | 2.4 | 2.0 |
| No aberrations (dead) | 7 | 5 | 1.0 | 1.0 |
| Total | 322 | 122 | 45.1 | 23.0 |

[a] The number pupated were 712 in ABG-6184 (starting with 2,500 larvae) and 529 in BSP-1 (starting with 1,500 larvae) in the treatment.
[b] Percentage based on the total number of pupae as in [a].

anomalies was higher in the ABG-6184 treatment than in the BSP-1 treatment. The total percentages of dead pupae (based on the pupal numbers) with aberrations were 45 and 23% for the 2 treatments, respectively. The higher percentage anomalies were albino pupae, larviform, black and enlarged distended pupae in the ABG-6184 treatment.

Adult males and females developing from the survivors of larvae treated at sublethal concentration of 0.002 mg/liter ($LC_{25}$) of ABG-6184 showed somewhat similar longevity as those in the check population (Table 5), except that the rate of death in females from the treated population was higher after the 3rd gonotropic cycle. Male mosquitoes were shorter lived than the females and died at about the same rate in the treatments and checks.

The fecundity of females among the treated and checks was essentially the same in each of the gonotropic cycles. With the progression of aging, there was a decrease in oviposition. During the first gonotropic cycle a mean of 0.98 and 0.93 egg rafts was laid per female in the treated and check regimen, respectively (Table 5). The mean number of egg rafts was only 0.33 and 0.37 for the treated and check, respectively, for the 6th gonotropic cycle.

The hatching rate of egg rafts in the treated and checks was essentially the same for each gonotropic cycle. The initial hatching rate was

Table 5. Posttreatment effects of *Bacillus sphaericus* (ABG-6184) on the longevity and fecundity of *Culex quinquefasciatus* adults resulting from larvae treated at 0.002 mg/liter ($LC_{25}$).

| Gonotropic cycle no. | Mean no. surviving[a] | | Mean no. egg rafts laid | Mean no. egg rafts per female | Mean no. egg rafts | |
|---|---|---|---|---|---|---|
| | Males | Females | | | Hatched | % hatch |
| 1st treated | 10.0 | 20.0 | 19.6 | 0.98 | 19.5 | 99.5 |
| Check | 10.0 | 20.0 | 18.6 | 0.93 | 18.0 | 96.8 |
| 2nd treated | 6.0 | 17.0 | 12.3 | 0.72 | 11.3 | 91.9 |
| Check | 7.0 | 19.0 | 13.3 | 0.70 | 12.5 | 94.0 |
| 3rd treated | 2.0 | 15.3 | 8.3 | 0.54 | 8.0 | 96.4 |
| Check | 3.0 | 18.0 | 11.3 | 0.62 | 11.0 | 97.3 |
| 4th treated | 0.0 | 11.6 | 8.0 | 0.69 | 7.0 | 87.5 |
| Check | 0.3 | 15.6 | 11.6 | 0.74 | 11.0 | 94.8 |
| 5th treated | 0.0 | 8.6 | 5.3 | 0.62 | 5.0 | 94.3 |
| Check | 0.3 | 15.0 | 12.0 | 0.80 | 10.6 | 88.3 |
| 6th treated | 0.0 | 3.0 | 1.0 | 0.33 | 0.7 | 70.0 |
| Check | 0.3 | 8.0 | 3.0 | 0.37 | 2.5 | 83.0 |

[a] Average of 3 replications. All except one male in check were dead after 3rd gonotropic cycle. Survivors from 1,500 larvae.

over 96% in the 2 regimens; however, it decreased somewhat in the subsequent gonotropic cycles, ranging between 87 and 97%.

Sublethal larvicidal concentrations of *B. sphaericus* had little or no impact on the fecundity and hatching rate of eggs laid by females developed from treated survivors. Similar lack of effects on sex ratio, fecundity and egg hatch of *Cx. quinquefasciatus* was noted with *B. sphaericus* strain 1593 (Mian and Mulla 1983). Effects on longevity of male and female mosquitoes were also insignificant, although a higher proportion of females in the treatment died in gonotropic cycle 4 through 6.

Lethal concentrations ($LC_{50}$ and greater) of *B. sphaericus* strain 2362 induced high to complete cumulative mortality including initial (48 h) as well as delayed. To study delayed mortality and extent of morphogenetic aberrations (in survivors consisting of larvae, pupae and adults), sublethal concentrations ($LC_{25}$ or lower) will have to be used. At the higher concentrations, most of the mortality occurs in the larval stage within the first 48–72 h of exposure. At the lower concentrations, some mortality, however, occurs in the larvae (see Table 1); but with large number of survivors, some attain the pupal and adult stages. It is in these succeeding stages (where larvae were treated at sublethal concentrations) that additional delayed mortality is realized and most of the dead specimens exhibit morphogenetic aberrations similar to those noted in mosquito larvae and resulting pupae and adults where the larvae were treated with insect growth regulators (Arias and Mulla 1975, Awad and Mulla 1984).

The most common and notable aberration in larvae was the formation of a transparent antero-dorsal thoracic bulbous projection (in 5% of the larvae, based on the total initial number). In these dead larvae the neck region was elongated, and the neck was swollen and enlarged. These types of deformities were reported by Awad and Mulla (1984) in larvae of *Cx. quinquefasciatus* treated with the IGR cyromazine.

Another prevalent and common occurrence was the failure of adults to eclose completely. These partially eclosed adults were unable to fly and all died. In these adults the wings were crumpled (either one wing or both), the mouth parts curled and appressed to the body and the legs also seemingly stuck together. In the process of eclosion, these adults could not successfully eclose from the pupal skin. In general, the adults eclosed only partially (just head and thorax freed) or almost completely but with the lower part of legs or tips of wings still attached to the pupal skin. All these adults whether partially or almost completely eclosed died. Some of the adults which eclosed completely had one or both wings crumpled. This feature was also a fairly common occurrence; the adults with such deformed wings were unable to fly, and they died on the water surface. Another common deformity in completely eclosed adults was the curling of the legs, even though the wings were of normal shape. These adults, as the other deformed adults, were unable to fly and died.

Sublethal concentrations of *B. sphaericus* 2362 induced additional mortality (beyond the 48 h exposure period) in the surviving larvae and succeeding stages of pupae and adults resulting from the larvae surviving the treatment. Also most of the individuals experiencing delayed mortality were afflicted with morphogenetic aberrations like those commonly found in

populations treated with IGRs. It seems that *B. sphaericus* preparations studied here have growth regulating compounds and properties related to the toxin particles or other parts of the cell. It is also conceivable that bioactive compounds may be contained in the formulation. Further detailed studies are warranted to elucidate the nature of these agents.

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