Toxicity of *Bacillus thuringiensis* β-exotoxin to Three Species of Fruit Flies (Diptera: Tephritidae)

JORGE TOLEDO,1 PABLO LIEDO,1 TREVOR WILLIAMS,1 AND JORGE IBARRA2


**ABSTRACT** The current study describes toxic effects of the *Bacillus thuringiensis* β-exotoxin toward 3rd instars of 3 fruit fly species: *Anastrepha ludens* (Loew), *A. obliqua* (Macquart), and *A. serpentina* (Wiedemann). The β-exotoxin was highly toxic to all 3 species tested, with LC₅₀ values calculated as 0.641, 0.512, and 0.408 μg/cm² of filter paper used to expose the larvae, for *A. ludens*, *A. obliqua*, and *A. serpentina*, respectively. Exposure to β-exotoxin was associated with an increase in the incidence of deformed pupae. The adult survivors from β-exotoxin treatments showed no negative effects in terms of their longevity, fecundity, or egg eclosion (fertility). We conclude that the β-exotoxin may have potential as a control agent for fruit fly pests.

**KEY WORDS** *Anastrepha* spp., bioassay, β-exotoxin, sublethal effects, Mexico, larvalcide

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**Materials and Methods**

Third-instar *Anastrepha ludens*, *A. obliqua* (Macquart), and *A. serpentina* (Wiedemann) were obtained from the Moscafrut production plant at Metapa, Chiapas in which cultures are maintained on a semi-synthetic diet as described by Dominguez et al. (1996), Moreno (1996), and Pinson et al. (1993), respectively. The β-exotoxin was obtained as experi
A. serpentina

1,110 0.408

A. obliqua

and 3.248

tested were 0.091, 0.152, 0.253, 0.421, 0.702, 1.169, 1.949,

Anastrepha distilled water in the case of the controls. Third-instar
disk impregnated with 1 ml of the toxin solution or
9-cm-diameter petri dishes containing a filter paper
(L:D) h, except where otherwise stated.

1,166 0.641

A. ludens

Institute 1992). The number of bioassays performed was 4 for
A. ludens, A. serpentina, and A. obliqua, respectively. These values
were significantly different between A. ludens and A. serpentina
(F = 7.18; df = 2, 8; P < 0.001) (Table 1).

Overall mortality in nontreated control insects (larvae + pupae) was low in all bioassays (never >11.94%). The results given in Table 1 were subject to
Abbott’s correction (Abbott 1925). There was no evidence of β-exotoxin contamination among different treatments at any stage.

The greatest effect of β-exotoxin treatment was evident in the pupal stage; the β-exotoxin appeared to interfere with pupation and adult emergence. The incidence of malformed pupae ranged from 0.83 to 5.0% but was not correlated with β-exotoxin concentration. In contrast, mortality at the larval stage was low (2.5–7.5%, depending on β-exotoxin concentration) and larval mortality in the controls was very low (0.55%).

Sublethal Effects of β-exotoxin in Adult Flies. There were no negative effects apparent in the longevity of A. ludens adults that had been exposed as larvae to β-exotoxin when compared with controls or among treatments (concentrations of β-exotoxin) (F = 1.25; df = 7, 139; not significant) (Table 2).

was recorded. A randomly chosen subsample of 100 eggs was placed on moist filter paper and held in a petri dish at 28 ± 0.5°C for 5 d, after which the percentage eclosion was checked and recorded.

Longevity in the cages was registered by noting the daily mortality of adult flies. Results were subjected to ANOVA followed by means separation using the LSD procedure (P ≤ 0.05) (SAS Institute 1992).

In the 2nd experiment, 3rd instars of each species were exposed to an LC50 concentration of β-exotoxin for 24 h and then transferred to damp vermiculite as previously described. Control larvae were treated with distilled water. When adults emerged, 10 females and 10 males of each species were confined in a cage with water and sugar-protein diet. Longevity, fecundity and egg viability of these flies was determined as described above. Data were analyzed by Student t-test of the means for each species separately (Steel and Torrie 1988).

Results

β-exotoxin Toxicity in Juvenile Stages. High levels of toxicity were detected in the bioassays with LC90 values of 0.641, 0.512, and 0.408 μg of β-exotoxin per square centimeter of filter paper for A. ludens, A. obliqua, and A. serpentina, respectively. These values were significantly different between A. ludens and A. serpentina (F = 7.18; df = 2, 8; P < 0.001) (Table 1).

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Table 1. Toxicity of B. thuringiensis β-exotoxin to 3 species of Anastrepha fruit flies

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>LC50 ± SE (μg/cm²) (range of 95% CI)</th>
<th>χ²</th>
<th>LC95</th>
<th>Equation of regression line</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. ludens</td>
<td>1,166</td>
<td>0.641 ± 0.064a (0.471-0.878)</td>
<td>3.392</td>
<td>4.694</td>
<td>Y = 5.424 + 2.157X</td>
</tr>
<tr>
<td>A. obliqua</td>
<td>840</td>
<td>0.512 ± 0.033ab (0.367-0.678)</td>
<td>3.366</td>
<td>2.560</td>
<td>Y = 5.701 + 2.433X</td>
</tr>
<tr>
<td>A. serpentina</td>
<td>1,110</td>
<td>0.408 ± 0.019b (0.293-0.466)</td>
<td>5.967</td>
<td>3.652</td>
<td>Y = 5.688 + 1.767X</td>
</tr>
</tbody>
</table>

LC90 values followed by the same letter are not significantly different (LSD test at 5% level).
The magnitude of this effect did not appear to be related to concentrations of toxin exposure. Significantly greater concentrations were not seen in the other species (Table 3). An almost identical result was obtained for the adult longevity of the adults emerging from nontreated larvae (Table 2). Unexpectedly, the fecundity of female survivors of the β-exotoxin exposure was significantly greater than that observed in nontreated flies, for all concentrations tested (\(F = 22.2\); \(df = 7.581\); \(P < 0.001\)). The magnitude of this effect did not appear to be related to β-exotoxin concentration. Egg eclosion (fertility) was also significantly greater in the surviving adults of the β-exotoxin- treated larvae compared with the adults emerging from nontreated larvae (\(F = 9.75\); \(df = 7.575\); \(P < 0.001\)). Eclosion of eggs was generally highest for the survivors of the low and moderate concentrations of β-exotoxin, being 11–32% higher than controls. Survivors of the 2 highest concentrations of β-exotoxin also had fertilities significantly higher than controls, although the difference was not as evident as in the other concentrations (Table 2).

After exposure of each species to an LC50 concentration of β-exotoxin, the longevity of adult flies was significantly greater in the survivors of toxin exposure than in controls for A. ludens and A. obliqua but not for A. serpentina (Table 3). Fecundity did not differ between control and β-exotoxin exposed survivors for any species. Egg eclosion was significantly higher in the survivors of β-exotoxin treatment for A. ludens (86.4%) compared with controls (80.4%), but this difference was not seen in the other species (Table 3).

### Table 2. Longevity, fecundity, and fertility of A. ludens adults obtained from larvae exposed to a range of concentrations of β-exotoxin

<table>
<thead>
<tr>
<th>Concentration of β-exotoxin, (\mu g/cm^2)</th>
<th>Mean longevity ± SE, days</th>
<th>Mean daily fecundity ± SE (eggs/female/day)</th>
<th>Mean % eclosion of eggs ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>66.6 ± 10.9a</td>
<td>14.95 ± 1.39e</td>
<td>34.36 ± 2.85f</td>
</tr>
<tr>
<td>0.091</td>
<td>56.2 ± 5.5a</td>
<td>43.40 ± 1.74a</td>
<td>66.00 ± 3.40a</td>
</tr>
<tr>
<td>0.152</td>
<td>79.5 ± 8.6a</td>
<td>28.06 ± 1.52e</td>
<td>58.08 ± 2.79b</td>
</tr>
<tr>
<td>0.253</td>
<td>69.7 ± 8.6a</td>
<td>27.42 ± 1.94c</td>
<td>43.94 ± 3.22d</td>
</tr>
<tr>
<td>0.421</td>
<td>57.7 ± 7.2a</td>
<td>33.70 ± 1.67b</td>
<td>52.04 ± 3.55bcd</td>
</tr>
<tr>
<td>0.702</td>
<td>65.7 ± 6.3a</td>
<td>34.17 ± 2.39b</td>
<td>54.63 ± 4.19c</td>
</tr>
<tr>
<td>1.169</td>
<td>56.5 ± 6.3a</td>
<td>24.63 ± 1.65c</td>
<td>41.21 ± 3.61ef</td>
</tr>
<tr>
<td>1.949</td>
<td>68.9 ± 8.1a</td>
<td>23.34 ± 1.98d</td>
<td>41.03 ± 3.62ef</td>
</tr>
</tbody>
</table>

Values in the same column followed by the same letter are not significantly different (LSD test at 5% level).

Discussion

The β-exotoxin of B. thuringiensis was shown to be highly toxic to larvae of Anastrepha fruit flies, and this effect was consistent among the 3 species tested: LC50 values differed by a factor of not >1.60. The differences, such as they were, may be related to the thickness of the epidermis of each species or to intrinsic physiological factors (Rabossi et al. 1991, Hopkins and Kramer 1992). In fact, the abnormalities observed in insects that died during the larval stage were highly variable, which may reflect differences in the physiological state, titers of ecdysonne, and other important hormones, at the moment of exposure to the β-exotoxin.

As the experimental larvae were exposed as 3rd instars, close to pupation, it is possible that more drastic symptoms of poisoning would have been seen had early instar larvae been used. Tanigoshi et al. (1990) reported that young nymphs of the bug Lygus hesperus Knight (Heteroptera: Miridae) were markedly more susceptible to topical applications of β-exotoxin than were late instar nymphs or adults.

The degree of toxicity observed in Anastrepha spp. larvae is comparable with that of other Diptera, Lepidoptera, and other pests insects. The LC50 of β-exotoxin in larvae of the fly Hematobia irritans (L.) was calculated at 2.79 \(\mu g/g\) of diet and symptoms of toxicity observed were similar to those seen in the current study (Haußer and Kunz 1985). In larvae of Helicoverpa zea (Boddie), H. cirescens (F.), Trichoplusia ni (Hübner), Pectinophora gossypiella (Saunders), and Spodoptera exigua (Hübner), adult emergence was totally prevented by a concentration of 0.540 \(\mu g/cm^2\) of larval diet although the stage at which most mortality occurred was not mentioned (Ignoffo and Gregory 1972).

The composition, origin and mode of action of the β-exotoxin of B. thuringiensis is totally different from the δ-endotoxins commonly employed as bioinsecticides. Previous studies of the use of B. thuringiensis against fruit flies have focused on the search for effective δ-endotoxins activity and to his end, the mosquitocidal subspecies B. thuringiensis ssp. israelensis has been shown to induce important levels of mortality in larvae and adult fruit flies, but only at very high doses (Yamvrias and Anagnost 1989, Robacker et al. 1996). Programs of screening of isolates for toxicity to

### Table 3. Adult longevity, fecundity, and fertility of 3 species of Anastrepha obtained from larvae exposed to their respective LC50 concentrations of β-exotoxin compared with control flies

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean longevity ± SE, days</th>
<th>Mean daily fecundity ± SE (eggs/female/day)</th>
<th>Mean % eclosion of eggs ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. ludens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated</td>
<td>74.9 ± 7.6a</td>
<td>46.6 ± 2.7a</td>
<td>86.4 ± 2.1a</td>
</tr>
<tr>
<td>Control</td>
<td>62.3 ± 7.2b</td>
<td>42.6 ± 2.1a</td>
<td>90.4 ± 1.9b</td>
</tr>
<tr>
<td>A. serpentina</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated</td>
<td>46.2 ± 6.1a</td>
<td>15.9 ± 0.8a</td>
<td>55.0 ± 4.6a</td>
</tr>
<tr>
<td>Control</td>
<td>35.5 ± 0.9a</td>
<td>16.1 ± 1.1a</td>
<td>57.1 ± 4.3a</td>
</tr>
<tr>
<td>A. obliqua</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated</td>
<td>30.5 ± 3.7a</td>
<td>24.2 ± 4.4a</td>
<td>91.2 ± 3.3a</td>
</tr>
<tr>
<td>Control</td>
<td>19.9 ± 3.7b</td>
<td>19.2 ± 2.9a</td>
<td>87.7 ± 1.7a</td>
</tr>
</tbody>
</table>

Pairwise comparison of means in the same column and for each species separately were performed by Student t-test. Values followed by the same letter are not significantly different at 5% level.
A. ludens and B. oleae have also been carried out with varying success (Karamanlidou et al. 1991, Bobacker et al. 1996), but in no case has the β-exotoxin content of these strains been tested; instead, attempts have been made to eliminate this substance from experimental formulations.

The results from the survivors of A. ludens from the bioassays and the tests of LC₉₀ exposure to all 3 Anastrepha species were for the most part consistent, with greater longevity, higher fecundity, and improved egg eclosion in the survivors of β-exotoxin exposure compared with control flies. This trend was not expected and contradicts the observations of others (Ignoffo and Gregory 1972, Sebesta et al. 1981). We suggest that exposure to the β-exotoxin eliminated the weakest individuals in the experimental population, leaving survivors that were, on average, more vigorous and reproductively superior to the control population.

However, this argument suggests that the survivors of the highest concentrations of β-exotoxin should have been the most vigorous and reproductively prolific, which was not, in fact, observed. Another possible explanation relates to the known inhibition of metamorphosis and new tissue formation by the β-exotoxin. The insect may respond to this inhibition by homeostasis of tissue formation, thus leaving proportionately more resources available for reproduction. Such ideas must, for the time being, remain speculative.

The adult survivors of β-exotoxin exposure did not show obvious signs of malformations, and there appeared to be no handicap to their successful mating and reproduction. This contrasts with 1 report in which lepidopteran survivors of β-exotoxin exposure showed antennal and buccal deformations that are likely to have direct effects on fecundity and survival (Ignoffo and Gregory 1972).

The β-exotoxin would appear to have clear potential for control of fruit flies, especially if applied to soil beneath the canopy of infested fruit trees. Nevertheless, the toxic effect of this substance extends to certain vertebrates, and many occidental countries have prohibited its use as an insecticide in its own right or as a contaminant in bioinsecticidal products based on the δ-endotoxins. Certain products, however, purposefully include the β-exotoxin, which allows a greater range of pest species to be effectively controlled than using the δ-endotoxin alone (e.g., Bitoxibacilin, available in Russia). The β-exotoxin has also been used in certain Nordic countries as a larvicide for control of flies in pig farms.

The 2 types of toxin may also interact synergistically resulting in significant improvement of the effectiveness of B. thuringiensis as a bioinsecticide against several important insect pests, including S. exigua (Moar et al. 1986). Moreover, it is clear that chemical products such as diazinon used for fruit fly control are highly persistent and have toxicity to a broad range of soil arthropods above and beyond that of the β-exotoxin of B. thuringiensis. Field studies now in progress aim to quantify the impact of β-exotoxin applied to the soil for Anastrepha control, in terms of direct mortality of the pest and the impact on beneficial soil fauna.

Acknowledgments

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