Toxicity of *Bacillus thuringiensis* var. *israelensis* to chironomids in pond mesocosms

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A pond mesocosm study was conducted in a central Minnesota wetland to evaluate the potential toxicity of the microbially-derived insecticide *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) to chironomids. *B.t.i.* was applied as VectoBac® G to mesocosms on two occasions (21 d apart) at five rates (0.3X, 1X, 2.5X, 5X, 10X) with three replicate mesocosms per rate. The 1X rate (9 kg/ha) was that operationally used by the Minneapolis-St. Paul Metropolitan Mosquito Control District for early summer mosquito control. Chironomid abundances following *B.t.i.* treatment were compared to abundances in untreated control mesocosms. The abundance of Chironomidae larvae was significantly reduced at the 10X treatment 4 d after the first *B.t.i.* application. Chironomid abundance was also reduced after the second application with 10X, but showed strong signs of recovery within 32 d. Chironominae, the numerically dominant subfamily within the Chironomidae, showed a similar response. The abundance of Orthocladiinae larvae was significantly reduced at both the 10X and 5X treatments, whereas the Tanypodinae appeared unaffected by all *B.t.i.* treatments. Of the two tribes comprising the Chironominae, the Chironomini displayed a response very similar to that of its parent subfamily, although reductions in abundance were not statistically significant. The tribe was dominated by *Dicrotendipes*, *Einfeldia*, and *Endochironomus*, none of which were significantly reduced following either 10X application. The second tribe, the Tanytarsini, were slightly more susceptible to *B.t.i.* than the Chironomini, displaying significant reductions in abundance after both 10X applications. The Tanytarsini were dominated by *Paratanytarsus*, which were reduced by 91% 4 d after both 10X *B.t.i.* applications. Tanytarsini and Chironomini were also reduced in abundance (by 83 and 75%, respectively) at the 5X treatment, but reductions were not statistically significant. Regressions of larval chironomid abundance versus *B.t.i.* treatment rate indicated that the *B.t.i.* rates required to reduce chironomid abundance by 25, 50, and 75% were 1.5–2.0X, 2.1–3.3X, and 3.5–11.0X, respectively. Emergence of adult Chironomidae was significantly reduced at the 10X *B.t.i.* treatment, but not at 5X. The same trend was observed for the Chironominae, which comprised 82% of the family, but not for Orthocladiinae and Tanypodinae. Emergence of Ceratopogonidae and Chaoboridae was unaffected by all *B.t.i.* treatments.

**Keywords**: *Bacillus thuringiensis* var. *israelensis*; toxicity; chironomids; wetland.

Introduction

Many compounds of bacterial origin have toxic properties that can be exploited to control the abundance of nuisance insects. One bacterium, *Bacillus thuringiensis* (*B.t.*), has long been known to produce proteinaceous, parasporal crystals during sporulation (Federici et al., 1990). These crystals are composed of diverse proteins, at least one of which is a protoxin (delta-endotoxin) that is activated (solubilized) in insects by alkaline intestinal pH and digestive enzymes (proteases). The parasporal crystals have no contact toxicity and must be ingested to exert their toxic action, which includes lysis and disintegration of midgut epithelial cells leading to death (Chilcott et al., 1990). Many different serovarieties of *B.t.* are known, most of which are active on Lepidoptera. One serovariety, *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*), is particularly active on Diptera of the suborder Nematocera. This unique selectivity, coupled with its operational efficacy, short
environmental half-life, and inexpensive production has resulted in the widespread use of \textit{B.t.i.} for mosquito and black fly control (Lambert and Peferoen, 1992; Priest, 1992). \textit{B.t.i.} has been used extensively since 1985 by the Minneapolis-St. Paul Metropolitan Mosquito Control District (MMCD) for mosquito and black fly control in southern Minnesota. The most commonly used formulation is VectoBac$^\text{H}$ G, a product consisting of \textit{B.t.i.} spores coated onto dried corncob granules. This formulation has been shown to be effective for reducing mosquito abundances in Minnesota wetlands, but it has also been found to impact nontarget chironomid populations in those wetlands (Niemi \textit{et al.}, 1995). In addition, experimental application of \textit{B.t.i.} to areas of the Mississippi and Minnesota Rivers in 1987–88 for black fly control revealed 70 and 78% mortality, respectively, of Tanypodini chironomids (Simmons, 1991).

Toxicity of \textit{B.t.i.} towards chironomids has also been reported outside of Minnesota for both lentic (Ali, 1981) and lotic (Back \textit{et al.}, 1985; Cilek and Knapp, 1992; Molloy, 1992) populations, and laboratory studies have shown that different species of chironomids respond differently to \textit{B.t.i.} (Garcia \textit{et al.}, 1980; Ali \textit{et al.}, 1981).

Although the susceptibility of some chironomid taxa to \textit{B.t.i.} has been recognized, no replicated field experiment has adequately established concentration-response relationships or ecotoxicological thresholds for these ubiquitous and ecologically important nontarget organisms. Furthermore, a recent study in a Minnesota wetland found no significant \textit{B.t.i.} toxicity to chironomids (Charbonneau \textit{et al.}, 1994), and most other recent field studies have focused on \textit{B.t.i.} effects on chironomids in lotic habitats following black fly control efforts (e.g., Molloy, 1992; Jackson \textit{et al.}, 1994; Wipfli and Merritt, 1994). The objective of this study was to describe the toxicity of \textit{B.t.i.} to chironomids in a central Minnesota wetland representative of those routinely treated by the MMCD for mosquito control. Emphasis was placed on determining the toxicological threshold application rates for \textit{B.t.i.} to naturally occurring populations. This included defining the no observed and lowest observed effect rates (NOER and LOER, respectively) for different chironomid taxa, and describing associated treatment rate-response relationships where possible.

\textbf{Methods}

\textit{Study site}

The study was conducted in a small (~0.8-ha) wetland located within the Larson Wildlife Production Area, Pope County, central Minnesota (45°, 25', 83°N; 95°, 21', 81°W). This wetland had a maximum depth of ~1.2 m and an encircling, well-developed zone of emergent vegetation dominated by cattails (\textit{Typha} sp.). Common bladderwort (\textit{Utricularia vulgaris} \textit{L.}) and decomposing pieces of cattails formed the majority of the submerged and floating vegetation mat. Giant duckweed (\textit{Spirodela polyrhiza} (L.) Schleid), water milfoil (\textit{Myriophyllum} sp.), and pondweed (\textit{Potamogeton} sp.) were also present, especially later in the summer. The percentage of each enclosure surface area that was covered by floating and emergent vegetation was estimated visually on five occasions against the partial grid created by the supports for the sampling disks. The maximum resolution was approximately ± 5%.

\textbf{Enclosure construction}

Eighteen enclosures were installed on June 7–8, 1995, on the east side of the wetland along the edge of the emergent vegetation zone. The surface area of each enclosure was 5.3 m$^2$ and the average water depth was 0.76 ± 0.03 m. The water depth varied by <0.1 m over the course of the study. Enclosures were constructed of 8.5 m long × 1.2 m wide pieces of 2 mm thick high density polyethylene, tightly secured at the ends to form a circular unit. Each enclosure was pressed ~15–25 cm into the sediment and secured between four pairs of 5 × 5 cm wooden posts (Fig. 1).

\textbf{B.t.i. application}

\textit{B.t.i.} was applied as VectoBac$^\text{H}$ G, a corncob granule (5/8 mesh size) formulation produced by Abbott Laboratories, Chicago, IL (Lot no. 04-587-N8; certified 200 ITU/mg). There were five application rates with three replicate enclosures per rate and three untreated controls. Treatments were allocated to enclosures following a randomized complete block design to compensate for possible habitat differences within the wetland. Each ‘block’ was composed of six adjacent enclosures. The five chosen \textit{B.t.i.} rates (0.3X, 1X, 2.5X, 5X, 10X) were multiples of the 8 lbs/acre (9 kg/ha) rate operationally used by the MMCD in early summer (1X). Preweighed amounts of granules were hand-

\begin{figure}[h]
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\includegraphics[width=\textwidth]{fig1.png}
\caption{Positioning of emergence traps and sampling disks within enclosures.}
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