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Research Article

Effect of *Bt* toxin on net-spinning caddisfly *Stenopsyche marmorata* (Trichoptera, Stenopsychidae)

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Abstract

The *Bacillus thuringiensis* (*Bt*) toxin targets lepidopteran, dipteran and coleopteran pests. Despite their close taxonomic relationship to Lepidoptera, few studies have examined the hazard of *Bt* toxins on Trichoptera. We performed laboratory exposure tests to examine the effects of *Bt* var. *aizawai* on filter-feeding caddisflies, particularly *Stenopsyche marmorata*. In the continuous exposure test, the mortalities ranged from 0% at 1 mg/l to 100% at 20 mg/l 24 hours after exposure to *Bta*. The mortality at each concentration increased from 24 to 48 hours. In limited contact, mortality increased with contact time. Acclimatisation and water quality also affected larval mortality. Our results suggest that higher concentration and longer exposure to *Bta* affect or affect the increase of larval mortality.

Key Words

Bacillus thuringiensis, filter feeding caddisflies, insecticide, larvae of *Stenopsyche marmorata*, mortality, pest control

Introduction

Bacillus thuringiensis (*Bt*) is a gram-positive bacterium found naturally in soil, water, dead insects and grain dust (Valicente et al. 2010). Amongst these, *Bt* is one the most effective microbial insecticide agent and its proteins are toxic to insects primarily belonging to the orders Lepidoptera, Diptera and Coleoptera (Domínguez-Arrizabalaga et al. 2020). *Bt* strains synthesise Crystal (Cry) and cytolytic (Cyt) toxins. Names for Cry toxins and their corresponding genes include a Roman numeral (primary rank distinction) depending on the insecticidal activity of the crystal protein, for example, CryI for proteins toxic to Lepidoptera (Palma et al. 2014).

The order Trichoptera, or caddisflies, is one of the major aquatic insect orders, which comprises a group of holometabolous insects closely related to the order Lepidoptera, Lepidoptera and Trichoptera being the two orders of Amphiesmenoptera (de Moor and Ivanov 2008). Larvae are vital participants in aquatic food webs and their presence and relative abundance are used in the biological assessment and monitoring of water quality (Holzenthal et al. 2007). From an ecological perspective, Trichoptera are important processors of organic matter in the river continuum concept (RCC) (Vannote et al. 1980). As processors of organic matter, collectively known as the functional feeding groups (FFG) of animals, they display the full array of feeding modes (Cummins 1973). Despite their close taxonomic relationship to Lepidoptera, few studies have examined the hazard of *Bt* toxins on shredders and scrapers caddisflies (Rosi-Marshall et al. 2007; Pott et al. 2020).

Stenopsyche marmorata Navás, 1920 is widespread in the southern part of the Eastern Palearctic Region, i.e.

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from Japan, Korea, Southern Kuril Islands and Sakhalin, while in Siberia it is limited to the southern regions, where the larvae inhabit rapid rivers. *Stenopsyche marmorata* larvae are filter-feeding detritivorous caddisflies. They are an important component of the zoobenthos in many watercourses and form a major link in trophic ecosystems of Eastern Palearctic mountain and streams. As their capture nets filter edible particles from the water, they aid in controlling the transport of organic material in streams. Due to their ecological significance and widespread distribution, filter feeding caddisflies are attracting the increasing attention of specialists (Kocharina 1999).

The objective of our study is to clarify the effect of *Bt* var. *aizawai* (*Bta*) on filter-feeding caddisflies, particularly *S. marmorata*.

Material and methods

Test organisms

Larvae of *Stenopsyche marmorata* were collected one day before the start of each experiment in spring 2022 from the middle reaches of the Tama River in Japan. The larvae were kept in aerated tap water and stream water collected from the source stream at 20 °C on a 14 : 12 light : dark cycle. River water from the larval sampling sites was also collected for use in the exposure test.

Effect of Bt toxin Cry1Ab on *Stenopsyche marmorata*

An experimental preparation of *Bta* (GC-91, active toxin 10%, 1000 B.m.H./mg, Jackpot, Arysta LifeScience Corporation, Japan) was used in this study. Crystallising dishes (φ12 cm × 6 cm) were filled with 400 ml tap water and 18 g glass beads (7 mm). Water flow was created by a magnetic stirrer at 300 rpm and the temperature was maintained at 20 °C using an incubator (Yokoyama et al. 2009).

Ten larvae were placed in each dish, and three replicates of each exposure duration and concentration combination were performed per experiment. One dish was always left untreated and used as control. As a non-acclimatisation test, a calibrated amount of *Bta* was added to the dishes (1 mg/l, 2 mg/l, 4 mg/l, 5 mg/l, 10 mg/l and 20 mg/l as active *Bt* toxin) and continuous and limited time exposure (30, 60, 120, 240, 480, 960 and 1440 minutes) tests were conducted. The *Bta* concentration from limited time exposure was only 20 mg/l. At the end of the limited time exposure, the larvae were removed from the dishes and immediately placed back into the dishes filled with tap water and glass beads. In addition, exposure tests with and without acclimatisation were conducted using tap water (Total Organic Carbon: 0.6 mg/l, July 2022, Narashino City 2022) and river water (Total Organic Carbon: 0.8 mg/l Haijima Bridge, 13 April 2022, Ministry of Land, Infrastructure, Transport and Tourism 2022) were used in this test. The acclimatisation was 12 hours before *Bta* exposure without any food and continuous exposure tests using river water were conducted. The *Bta* concentrations in this study were 1 mg/l and 10 mg/l.

No food was provided during the experiment. The samples were observed at 24 and 48 h of the experiment. Larvae that were presumed dead were probed for possible movement with tweezers and data on the number of dead and live larvae in each dish was recorded.

Results

In the continuous exposure test, all larvae were dead within 24 hours at 20 mg/l (Fig. 1). The mortality at each concentration increased from 24 to 48 hours (Fig. 1). In addition, all of the individuals died without net building at 20 mg/l.

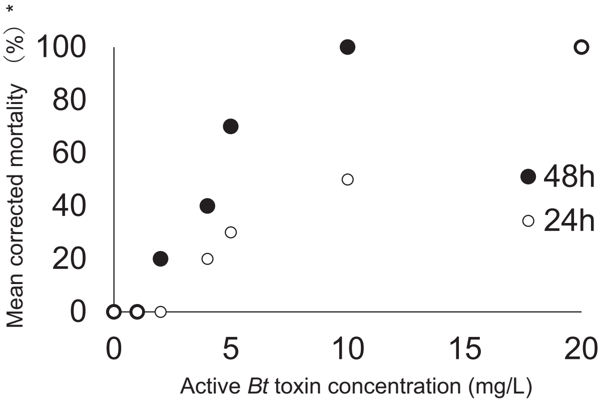

Figure 1. Mean corrected mortality (%) of *S. marmorata* 24 and 48 hours after exposure to *Bta*. *Mortality adjusted by Abbott's method (1925) with respect to elapsed time after exposure.

Under limited time exposure, dead individuals appeared at 480 minutes and mortality increased with contact time (Fig. 2). In contrast, there was no difference in mortality rate at each exposure time between 24 and 48 hours.

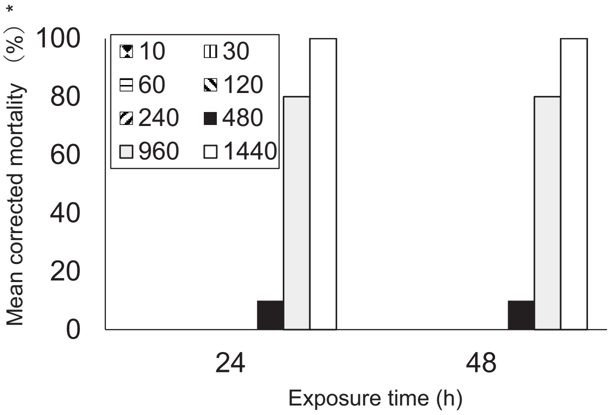

Figure 2. Mean corrected mortality (%) of *S. marmorata* 24 and 48 hours after limited time exposure to *Bta*. *Mortality adjusted by Abbott's method (1925) with respect to elapsed time after exposure.

In addition, larvae formed capture nets during the acclimatisation. The highest mortality was in the combination of acclimatisation and river water. At 10 mg/l, the mortality rate after 48 hours was high in each combination. At 1 mg/l, the mortality rate for the combination of river water and acclimatisation was 30%. Moreover, the larvae did not die under non-acclimatisation and tap water.

Discussion

In the present study, the effect of *Bta* on filter-feeding caddisfly, *S. marmorata* high concentration and longer exposure to *Bta* affected larval mortality. The 20 mg/l that was effective in this study is the concentration at which this *Bta* product is used on *Spodoptera frugiperda* (Lepidoptera, Noctuidae) in the field (Arysta LifeScience Corporation 2022). Our results indicate that short term contact (< 480 minutes) with *Bta* at a concentration of 20 mg/l does not induce mortality in experimental settings. In addition, acclimatisation and water quality (river water or tap water) also effected larval mortality.

Spores created by *Bt* damage the gut of insect larvae after they are consumed by the larvae, meaning the *Bt* is effective when eaten. Several studies demonstrated sublethal effects on caddisfly larvae after exposure to Bt toxins (Chambers et al. 2010; Jensen et al. 2010). Our results suggest that the silken net to capture food and the presence of suspended solids in the water enable larvae to rapidly feed on *Bta*.

Trichoptera are major pests in certain parts of Japan as the mass emergence of the adults can cause the obstruction of business (Kobayashi and Takemon 2014) and cause of bronchial Asthma (Kino and Oshima 1978). Furthermore, the larval nets and net-spinning caddisflies impede the water flow of hydroelectric power plants (Fujinaga and Sakaguchi 2005). *Bt* toxins target specific insect orders (Bravo et al. 2007). Jackson et al. (2002) examined the effects of the microbial pesticide *Bt israelensis* (*Bti*) on pestiferous black flies (primarily the *Simulium jenningsi* group), as well as non-target macroinvertebrates and fish. They found no evidence that a single *Bti* application affected non-target macroinvertebrates and fish to an ecologically significant extent. *Bti* has been successfully introduced for the management of nuisance Simuliidae and Chironomidae in Japanese rivers and streams (Miyazaki et al. 1998; Ogata and Sasa 1999). Our results indicate that *Bta* is a potential method to reduce trichopteran pests in Japan.

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