Marine Environmental Research 83 (2013) 29–37

Contents lists available at SciVerse ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

Eco-physiological response of two marine bivalves to acute exposition to commercial Bt-based pesticide

B. Manachini [a], V. Arizza [a], A. Rinaldi [b], V. Montalto [b], G. Sarà [b,*]

[a] Dipartimento di Biologia ambientale e Biodiversità, Università degli Studi di Palermo, Italy
[b] Dipartimento di Scienze della Terra e del Mare (DiSTeM), Università degli Studi di, Viale delle Scienze Ed. 16, 90128 Palermo, Italy

ARTICLE INFO

Article history:
Received 24 July 2012
Received in revised form
17 October 2012
Accepted 21 October 2012

Keywords:
Bacillus thuringiensis
Mytilaster minimus
Brachidontes pharaonis
Biological pollution
Biopesticides
Alien species
Agricultural coastal areas

ABSTRACT

Microbial products based on the entomopathogenic bacterium *Bacillus thuringiensis* (Bt) are among the most common biopesticides used worldwide to suppress insect pests in forests, horticulture and agricultural crops. Some of the effects of commercial Bt have been recorded for terrestrial and freshwater non-target organisms but little research is available on marine fauna. Nevertheless, due to the contiguity of agro-ecosystems and coastal habitats, marine fauna may be highly influenced by this control method. We studied the effect of a commercial Bt product on the physiological and ecological responses and the energy budget of two of the most frequent marine intertidal bivalves in the Mediterranean, the native *Mytilaster minimus* and the invasive *Brachidontes pharaonis*. To test the effects experimentally, we simulated the worst scenarios possible using the average dose applied to fields and a hypothetical accumulation dose. The results showed the feeding rates of both species were affected detrimentally by the different experimental conditions; higher concentrations led to higher respiration rates, however neither species showed any significant difference in excretion rates. The biopesticide had a significant effect on the energy budget, the values decreasing with doses. In addition, it led to high mortality for the worst treatments and, in both species, induced significantly higher cardiac activity than in the controls. These results indicate a measurable effect of Bt commercial products on marine organisms, and great attention should be paid to biopesticides composed by entomopathogenic bacteria and addictive compounds. In addition, the results highlight the urgent need to study not only the effects of anthropogenic pressures on target organisms but also to extend our view to other ecosystems not expected to be influenced. Gaining data at the organismal level should help increase the sustainability of pest control and reduce the consequences of side-effects.

1. Introduction

The World Health Organization (WHO), the Food and Agriculture Organization (FAO) and other International Organizations are now calling for the development of environmentally sustainable systems that are less reliant on chemical pesticides as the primary management tools for pest control (FAO, 2010; Manachini, 2012).

In recent years, significant progress has been made in the development of biocontrol agents for the suppression of pests (e.g., insects, nematodes, molluscs), weeds and diseases impacting a wide range of forest, horticultural and agricultural crops (Laengle and Strasser, 2010; Manachini, 2012). Among biocontrol tools, microbial pesticides are generally regarded as posing lower risks to human health and the environment than chemical pesticides (OECD, 2007). Microbial pesticides are based on the entomopathogenic bacterium *Bacillus thuringiensis* (Bt), and are used worldwide for the control of lepidopterans and coleopterans (Manachini et al., 2011), and against aquatic larvae dipteran pests (Laengle and Strasser, 2010).

However bacteria and fungi used in biological control have, under certain conditions, the potential to act as opportunistic pathogens, infecting species not normally susceptible to these organisms (Laengle and Strasser, 2010). In fact, there is some evidence that also microbial agents can also infect other disease-causing living organisms.

Non-target organisms present in agro-ecosystems and nearby areas, including coastal areas, can also be exposed to Bt in several ways including, for example, through feeding, transfer through the food chain, or by direct contact. Thus, the assessment of procedures for potential Bt impact on non-target marine organisms should be addressed in the context of biodiversity conservation. Many studies

* Corresponding author.
E-mail address: gianluca.sara@unipa.it (G. Sarà).

0141-1136/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.marenvres.2012.10.006

have investigated the potential effects of Bt toxins expressed in genetically modified plants and Bt commercial products mainly on non-target terrestrial fauna (Boisvert and Boisvert, 2000; Pouline et al., 2010) while there is little data on effects of marine organisms (Cervino et al., 2006; Eder and Schönbrunner, 2010). Some authors (Boisvert and Boisvert, 2000; Eder and Schönbrunner, 2010) have proposed the idea that accidental overspray or runoff containing commercial Bt could contribute to unexplained diseases in coral reef invertebrates. In addition, a possible effect of the endotoxin-producing *Bacillus* spp. was suspected in corals, sponges (Negri et al., 2009) and the marine worm *Nereis* (*Hediste*) *diversicolor* (Fourcy et al., 2002). Duchet et al. (2010) suggested a possible delayed effect of Bt-treatment in mesocosm on growth of *Daphnia magna*. Nevertheless no statistical significant correlation was found between Bt concentration and the mortality or longevity of shrimps (Eder and Schönbrunner, 2010).

Bt has also been isolated from marine habitats (Yin-Jjuan et al., 2008) and it is known that, in seawater, less than 10% of Bt (var. *kurstaki*) cells survived for 40 days after inoculation. Nevertheless, the activity of the viable spores, parasporal bodies and endotoxin in aquatic and more specifically marine habitats has not yet been definitively assessed. Only incomplete and contradictory data are available about the *Bacillus* spp. persistence in seawater (Menon and de Mestral, 1985; Surgeoner and Farkas, 1990; Tilquin et al., 2008; Van Cuyk et al., 2011).

While about 80% of the Earth's biodiversity is found in marine environments, the effect of pesticides on marine organisms remains relatively neglected (Callow and Willingham, 1996). Moreover, biomarkers commonly used to assess the impact on non-target marine organisms are not fully appropriate to explain potential direct and indirect effects. Thus further investigations are pressing (Fourcy et al., 2002), and reliable models for this task could be limpets, winkles, mussels, crabs and fishes (Clynick et al., 2009; Dondero et al., 2011; Gagnaire et al., 2006) living in supratidal and intertidal habitats (where the terrestrial meets the marine realm; Sarà et al., 2012b). Due to their proximity, these habitats are particularly exposed and vulnerable to active substances coming from adjacent terrestrial habitats. Here, we have chosen two bivalve species as experimental models to study the effect of Bt in a marine environment. Bivalves are among the most important ecosystem engineers in aquatic habitats (Gutierrez et al., 2003). Their role in ecosystems is particularly attractive, and they can be used as tools to study global ecological processes and to assess the effects of natural and anthropogenic stress in marine ecosystems (Sarà et al., 2011, 2012a). Indeed, marine bivalves are ubiquitous sessile animals able to play a key-role in modifying and structuring worldwide intertidal/subtidal habitats (*sensu* Bayne, 2004; Gutierrez et al., 2003; Jones et al., 1997; Manganaro et al., 2009; Sousa et al., 2009).

We focus on the effect of a biological insecticide used for Mediterranean crops on physiological responses of two intertidal Mytilids, the Lessepsian *Brachidontes pharaonis* (Fischer, 1870) and the autochthonous *Mytilaster minimus* (Poli, 1759). As no data are available on the susceptibility of these non-target organisms and data on exposure and concentration to Bt in seawater are scant and contrasting, we considered the two worst scenarios possible, using two high concentrations of commercial Bt. Thus, under laboratory mesocosmal conditions we measured, in both species, i) feeding rates, as expressed by clearance and ingestion rates, ii) oxygen consumption and excretion rates, iii) assimilation and absorption rates, iv) energy available for growth and reproduction (i.e., Scope for Growth, SFG – Widdows and Staff, 2006) and, lastly, heart beat rate, a reliable measure of stress successfully adopted in recent studies with other marine invertebrates (Dong and Williams, 2011; Halldórsson et al., 2007).

2. Material and methods

2.1. Study area, sampling and experimental set-up

Adults of *B. pharaonis* (mean total length = 2.0 ± 0.5 cm) and *M. minimus* (mean total length = 1.5 ± 0.2 cm) were collected by hand from two different intertidal areas on June 16 and 17, 2010: *B. pharaonis* from the Stagnone di Marsala Lagoon (Western Sicily, Southern Tyrrhenian; 37° 52′ N–12° 28′ E) and *M. minimus* from Altavilla Milicia, close to Palermo (Western Sicily, Southern Tyrrhenian; 38°3′ N–13° 33′ E). Once collected, the specimens were brought back to the Laboratory of Experimental Ecology and Behaviour (Palermo, Italy) and were acclimated at standard laboratory conditions (20 ± 1 °C and 37 ± 1‰) in large 400-L tanks and fed with a monoalgal culture of *Isochrysis galbana* (ad libitum; Sarà et al., 2008). According to the common experimental procedures (Ezgeta-Balic et al., 2011; Sarà et al., 2008; Widdows and Staff, 2006) previously adopted with success in studying the bioenergetics of bivalves, the animals of both species were kept under acclimation conditions for two weeks. At the end of the acclimation period, we randomly collect 240 animals of each species; they were divided into six groups of 40 specimens and transplanted in six 10-L aquaria. Thus, we had 6 aquaria containing 40 specimens each ($n = 6 \times 40$, i.e., 240) of the two species, ready to be inoculated with Bt as described below. The inoculum used was prepared from a commercially available insecticide based on *B. thuringiensis* var. *kurstaki* H-3A, 3b EG 2424 (hereafter called Bt). The commercial product was applied as a suspension concentrate formulation containing 71.2 g of active product per litre (Bt as spores and crystal toxins). The treatment rate was 3.5 L/ha, which would be the mean presumed recommended rate for field applications (field dose, FD). A second dose was chosen to simulate a possible concentration and accumulation effect (accumulation dose, AD). Thus the concentrations applied to mesocosms were 45 and 90 μL L^{-1}, respectively (AD and FD corresponding to 1.51×10^5 and 3.2×10^5 CFU ml^{-1}, respectively). Accordingly, we inoculated 2 aquaria with field dose – FD, 2 tanks with accumulation dose – AD and two aquaria were not treated with Bt and thus considered as control (here after called CTRL). This was performed for both species; the experiment consisted in measuring eco-physiological rates after 24 h of Bt exposure according to the following classical procedures.

2.2. Physiological measurements

After 24 h from treatment with different concentrations of Bt, as described above, we measured the following eco-physiological rates: clearance rate, respiration rate, food absorption efficiency and excretion rate using procedures described by Widdows and Staff (2006) and successfully applied by Sarà et al. (2008) and Ezgeta-Balic et al. (2011). In addition, heart beat rate in each bivalve species was measured by following procedures employed by Depledge and Anderson (1990), Halldórsson et al. (2007) and Sarà and De Pirro (2011). To measure the eco-physiological responses of *B. pharaonis* and *M. minimus*, we used eight individuals ($n = 8$) for each species collected from each 10-L aquarium where they were treated with Bt. Clearance rates were individually measured in a closed system by placing one individual each in a beaker containing 1 L of filtered thermo-regulated seawater (20 °C). The beakers were positioned on heated stirring base plates that ensured constant temperature and kept the water mixed and oxygenated throughout the experimental sessions. After a period of 20 min, as the bivalves started to filter, algal cells (*I. galbana*) were added to each beaker at an initial concentration of 25,000 cells ml^{-1}. Twenty ml aliquots were sampled from every beaker at 30 min intervals over a period of 2 h. The decline in *I. galbana* cell concentration was