

RAPID COMMUNICATION

Susceptibility of *Hypsipyla grandella* (Lepidoptera: Pyralidae) to *Bacillus thuringiensis* strains

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Abstract

The use of *Bacillus thuringiensis* (*Bt*) to control insect pests has already been established in various agronomic and forest crops. It is a bacterium that does not pollute the environment, is safe for mammals and vertebrates, lacks toxicity to plants and specifically targets insects. To date in-depth studies have not been conducted about the use of *Bt* to control the main pest of mahogany (*Swietenia macrophylla* King) and other Meliaceae species, the *Hypsipyla grandella* Zeller (Lepidoptera: Pyralidae). Therefore, this study aimed to test the pathogenicity of *Bt* strains on *H. grandella* caterpillars, as well to determine the lethal concentration required to kill 50% of the population (LC_{50}) of the most promising strains. Ten strains of *Bt* toxic to lepidopteran proven in previous trials were used and these were incorporated into a natural diet with mahogany seeds to check their mortality. The LC_{50} of the top five strains was determined. The results indicate that *H. grandella* is highly susceptible to *Bt* toxins and the S1905 strain is highly toxic. Therefore, the use of *Bt* strains may be a tool to be incorporated into the integrated management of this important pest.

Key words: *Bacillus thuringiensis*, bioassays, biocontrol, forest pest, mahogany shoot borer

Introduction

Hypsipyla grandella Zeller (Lepidoptera: Pyralidae) is an important pest of 13 genera within the Meliaceae family. In Brazil, this insect attacks mahogany (*Swietenia macrophylla* King), cedar (*Cedrella odorata* L.) and andiroba (*Carapa guianensis* Aubl.). *Hypsipyla grandella* attacks branches, shoots, leaves, fruits, bark and even roots (Yamazaki *et al.* 1990; Taveras *et al.* 2004), however, the main damage in mahogany consists of the destruction of the terminal shoot in seedlings and young trees, due to the entrance and excavation of tunnels by the caterpillars (Grijpma 1976). The symptoms of caterpillar attack are exudation of gum and sawdust, the presence of dry leaves in the middle of green foliage, and issuance of new shoots at each consecutive attack, which will also be attacked later (Silva 1985; Griffiths 2000). The growth of a straight trunk is strongly impaired (Grijpma 1976), with loss in height of up to

35% in the early years (Ohashi *et al.* 2005). Repeated and intense attacks can cause plant death.

The main methods usually adopted to control this pest in Brazil are: silvicultural management through interference in host plant location, use of insect resistant genotypes, reduction of host adequacy, increase in natural enemies, recovery of shape and increase of plant height, use of semiochemicals, and biological control through the use of fungi, wasps, nematodes and bacteria (Lunz *et al.* 2009). The use of conventional insecticides has been inadvisable for the control of this pest for reasons such as insect habit (cryptic), the nature of damage (internal to the plant), climatic factors (intense rainfall in the region of natural occurrence) and the long period of protection required, which makes it costly, impractical and damaging to the environment (Wylie 2001; Mahroof *et al.* 2002).

A possible alternative to control *H. grandella* is the use of biological agents, like fungi (Castro *et al.* 2017) and bacteria. *Bacillus thuringiensis* Berliner is a bacterium that produces proteins toxic to various insect orders, including forest pests and disease vectors for humans and animals (Baum *et al.* 1999). The advantages of using this microorganism are its high specificity to vulnerable insects, its non-polluting effect on the environment, its innocuousness to mammals and vertebrates and the absence of toxicity to plants (Whiteley and Schnepf 1986).

Currently, there are no strategies to effectively control the incidence of *H. grandella* in commercially important Meliaceae. Therefore, the aim of this work was to test the pathogenicity of *B. thuringiensis* strains to *H. grandella* caterpillars.

Materials and Methods

Strains used

Ten strains of *B. thuringiensis* (S602, S1264, S1289, S1301, S1905, S1979, S2021, S2122, S2124, S1450) belonging to the Invertebrate Bacteria Collection of Embrapa Genetic Resources and Biotechnology were selected for their toxicity to Lepidoptera, and because of their differing *cry* gene compositions (Praça *et al.* 2012; Macedo *et al.* 2012).

Tests of toxicity

Two types of bioassays were done. First, a selective bioassay, to select strains that caused more than 90% mortality, and later, a dose bioassay, to determine the dose necessary to kill 50% of larvae exposed.

Selective bioassay

The strains were cultured in 2-liter Erlenmeyer flasks containing 600 ml of Embrapa medium (Monnerat *et al.* 2007) in a rotary incubator at 200 rpm, 28°C, for 72 h until complete sporulation, when viewed under an optical phase contrast microscope with magnification of 1,000× for spores and crystals. The bacterial broth (100 µl) was mixed with ground mahogany seeds in a sterile Petri dish and offered to 10 neonate caterpillars (1st instar) per dish, with three replicates. The caterpillars were obtained by mass rearing in the Embrapa Insect Rearing Platform (Castro *et al.* 2016). The negative control consisted of the ground mahogany seeds mixed with distilled water. The material was housed in the Biological Oxygen Demand (BOD) with a photoperiod of 12 h, at 25 ± 2°C. Mortality was assessed after 48 h.

Dose bioassay

Strains that caused more than 90% mortality were again cultured and after 72 h were centrifuged at 9,500 rpm for 30 min (Hettich Zentrifugen Centrifuge, model Rotanda 460R). The pellet was resuspended in distilled water, frozen and then lyophilized in a Christ Freeze Dryer, Model Alpha 2–4 LD Plus. The lyophilized material was packed into Falcon tubes and stored at –20°C. The spores contained in the lyophilized material were quantified by the number of colony forming units per ml (CFU · ml⁻¹) using serial dilutions (Monnerat *et al.* 2007). The calculation of the lethal concentration to kill 50% of the individuals (LC₅₀) was determined from four serial dilutions of lyophilisate from the selected strains, which were then placed in ground mahogany seeds. For each dilution, 10 neonate caterpillars (1st instar) were used, with three replicates. The negative control consisted of the ground mahogany seeds mixed with distilled water. Mortality assessment occurred after 48 h and live caterpillars were transferred to another Petri dish containing untreated ground seeds. To do this, a stock solution was first prepared with 0.5 mg of the bacterial lyophilisate plus 5 ml of autoclaved distilled water. From the stock solution, serial dilutions were made by withdrawing 0.5 ml of the solution and placing it in 4.5 ml of autoclaved distilled water, and repeated, until the dilution was 10⁻⁹. The concentrations used in the bioassays were 2,000, 432, 93 and 20 ng · cm⁻².

The LC₅₀ was determined by Probit analysis (Finney 1971), with the aid of the Polo-Plus® program. The confidence intervals (95%) for LC₅₀ were calculated and non-overlapping of this parameter was used to detect significant differences between strains.

Results and Discussion

All of the 10 strains used were highly lethal to *H. grandella*, but five that caused mortality in more than 90% of *H. grandella* larvae were selected for further study (Table 1). The *cry* genes present in these strains were determined in prior studies (Macedo *et al.* 2012; Praça *et al.* 2012) and are shown in Table 1.

The dead larvae in the bioassays presented flaccid bodies and brown to dark brown integuments, with an opaque appearance. Some larvae were slow and had difficulty moving, symptoms characteristic of *Bt* infection (Habib and Andrade 1998; Praça *et al.* 2012). An important observation was related to the time of death, in that strains 2124, 1905 and 1450 caused death faster than the other treatments.

The LC₅₀ of the best strains (S1905, S2021, S2122, S2124 and the standard *Btk* 1450 HD-1) varied between 9.55 and 85.61 ng · cm⁻² (Table 1). The standard

Table 1. Larval mortality of *Hypsipyla grandella* by *Bacillus thuringiensis* strains after 24, 48 and 96 hours and lethal concentrations (LC₅₀) of *B. thuringiensis* strains to kill *H. grandella* larvae in a population tested 96 hours after treatment

Strain	Mortality [%]			cry genes	Strain	LC ₅₀ [ng · cm ⁻²]*	Confidence interval [95%]
	24 h	48 h	96 h				
S602	80	86.6	86.6	<i>cry1Aa, cry1Ab, cry1Ac, cry2Aa</i>	S1905	12.715 a	0.452–40.318
S1264	70	76.6	76.6	<i>cry1Aa, cry1Ab, cry1Ac, cry2Aa</i>	S2021	85.617 a	11.731–244.778
S1289	60	63.3	70	<i>cry1Ab, cry1Ad</i>	S2122	20.573 a	0.197–74.360
S1301	73.3	80	80	<i>cry1Ab, cry1F, cry1Ac, cry1D, cry1G, cry2Aa, cry2Ab</i>	S2124	27.520 a	1.016–87.713
S1905	93.3	100	–	<i>cry1Aa, cry1Ab, cry1Ac, cry1B, cry2Aa</i>	Btk 1450 HD-1	9.554 a	0.097–36.167
S1979	60	60	66.6	<i>cry1Aa, cry1Ab, cry1Ac, cry1Ad, cry2Aa, cry2Ab</i>			
S2021	83.3	86.6	100	<i>cry1Aa, cry1Ad, cry2Ab</i>			
S2122	90	100	–	<i>cry1Aa, cry1Ab, cry1Ad, cry1C, cry1D cry1F</i>			
S2124	86.6	100	–	<i>cry1Ab, cry1E, cry2Aa, cry2Ab</i>			
Btk 1450 HD-1	100	100	–	<i>cry1Aa, cry1Ab, cry1Ac, cry1B, cry2Aa cry2Ab</i>			
Control	6.66	10	16.6	–			

*means followed by the same letters do not differ by confidence interval according to Probit analysis

strain Btk 1450 HD-1 presented the lowest concentration (9.55 ng cm⁻²), while the S2021 strain presented the highest (85.61 ng cm⁻²). However, they did not differ statistically because the confidence intervals overlapped, which indicates that the strains are equally effective in the control of *H. grandella*.

The susceptibility of first instar *H. grandella* larvae to *Bt* was previously demonstrated by Hidalgo-Salvatierra and Palm (1973), in which the authors obtained up to 100% mortality using different dilutions of a single bacterial strain, proving that the insect is highly susceptible to the bacterium, corroborating the results of the present study. However, the LC₅₀ of the strain used was not estimated. Goulet *et al.* (2005) used the product DiPel 6.4 WG (*B. thuringiensis* var. *kurstaki* 6.4%, AB-BOTT Laboratories, Chicago) in spray form to control *H. grandella* in plantations of *Swietenia humilis* Zucc. and only 17% of the trees were attacked, compared to 44% of control plants without the bacterium.

In Brazil, the main use of *B. thuringiensis* in forestry occurs to control *Thyrntina arnobia* Stoll (Lepidoptera: Geometridae), the most important defoliator pest of *Eucalyptus* spp. plantations (Zanuncio *et al.* 1992; Agrofit 2017). Applications of *B. thuringiensis* against forest pests have effectiveness in ultralow volumes which increase the concentration of toxin ingested by the insects (van Frankenhuyzen 1990). The use of the most powerful strains in this work (Table 1) may be promising to control *H. grandella* when applied before the damage occurs, in the first instar larvae (Hauxwell *et al.* 2001), and with using strains which have different

compositions of *cry* toxins. The cryptic nature of the insect makes it difficult to control, but the use of sprays with *B. thuringiensis* strains may be used in an integrated pest management (Goulet *et al.* 2005).

The results indicate that *H. grandella* is highly susceptible to *B. thuringiensis* toxins and the use of this bacterium may be incorporated into the integrated management of this important pest of Meliaceae species.

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