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Control of mahogany shoot borer, *Hypsipyla grandella* (Lepidoptera: Pyralidae), with *Bacillus thuringiensis* in a systemic way

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ABSTRACT: *Hypsipyla grandella* (Lepidoptera: Pyralidae), popularly known as the mahogany borer, is the main pest of *Swietenia macrophylla* (Meliaceae), limiting the commercial planting of the species throughout Brazil. The use of the *Bacillus thuringiensis* (Bt) bacterium for the control of insect pests is becoming a promising tool to be incorporated into integrated pest management programs for various agricultural and forest pests. To date, no in-depth studies on the use of Bt for control of *H. grandella* have been conducted. Therefore, this study aimed to evaluate the systemic use of Bt in mahogany seedlings for insect control. The results demonstrated that plants treated with the S1905 strain showed a decelerated attack compared with the control, with little apparent damage or symptoms. This work is an early effort in the systemic use of Bt in seedlings of tree species and the method seems to offer a promising and viable alternative to the use of chemical insecticides.
Keywords: endophytic; cry toxins; forest pest; forest entomology; biological control.

Controle da broca-do-mogno, *Hypsipyla grandella* (Lepidoptera: Pyralidae), com o uso sistêmico de *Bacillus thuringiensis*

RESUMO: *Hypsipyla grandella* (Lepidoptera: Pyralidae), conhecida popularmente como broca-do-mogno, é a principal praga de *Swietenia macrophylla* (Meliaceae), limitando o plantio comercial da espécie no Brasil. O uso da bactéria *Bacillus thuringiensis* (Bt) para o controle de insetos pode se tornar promissor para ser incorporado em programas de manejo integrado de pragas de várias pragas agrícolas e florestais. Não há, atualmente, estudos com o uso de Bt para controlar *H. grandella* de forma sistêmica. Assim, esse estudo teve como objetivo avaliar o uso sistêmico de Bt em mudas de mogno para o controle do inseto. Os resultados demonstraram que as plantas tratadas com a estirpe S1905 mostraram um ataque desacelerado quando comparado com o controle, com poucos danos e sintomas. Esse trabalho é pioneiro no uso sistêmico de Bt em mudas de espécies florestais e esse método pode ser uma alternativa viável e promissora ao uso de inseticidas químicos.
Palavras-chave: endofítico; toxinas cry; praga florestal; entomologia florestal; controle biológico.

1. INTRODUCTION

Mahogany (*Swietenia macrophylla* King) is a forest species with high commercial value in the world, due to the quality and beauty of its wood, being considered as noble or “hardwood” in many countries (CARVALHO, 2007). *S. macrophylla* cultivation is established in more than 40 countries around the world, mainly in the humid and subhumid areas of the tropics in South and Southeast Asia, the Pacific Islands, the Caribbean and tropical Africa (CABI, 2005). According to Pandey (1983), in 1980 there were 55,000 hectares of *S. macrophylla* plantations in the world. Already in 1995, the plantations of the species covered approximately 151,000 hectares (PANDEY, 1997). In Indonesia, there are more than 55,000 hectares planted (COSSALTER; NAIR, 2000) and in the Fiji Islands approximately 26,000 hectares (KAMATH et al., 1996), thus being the countries that most plant mahogany. In Brazil, there are no data about the exact number of mahogany plantations, only some reports of cultivation in the North of the country, mainly in Pará state, and Central-West, mainly in Goiás, Minas Gerais and São Paulo states. The cultivation of mahogany outside its area of origin was considered a failure due to growing conditions, low quality seedlings and incidence of mahogany shoot borer, *Hypsipyla grandella* Zeller (Lepidoptera: Pyralidae) (CARVALHO, 2007).

The symptoms of *H. grandella* caterpillar attack are exudation of gum and sawdust, 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, OHASHI et al., 2002). The growth of a straight trunk is strongly impaired (GRIJPMA, 1976; OHASHI et al., 2000), 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 insect can cause damage in fruits of mahogany too (CASTRO et al., 2018a).

In a review by Lunz et al. (2009), the main methods studied for shoot borer control are the use of genotypes resistant to the insect; forestry management by interference in the location of the host plant, reduction of host suitability, increase in natural enemies and increase in plant height; use of semiochemicals; and biological control, with the use of fungi, wasps, nematodes and bacteria. The use of conventional insecticides has been inadvisable to control shoot borer for reasons such as the insect's habit (cryptic), the nature of the damage (internal in the plant), weather (heavy rainfall in the naturally occurring region) and the long period of protection necessary that endures almost all of the plant life cycle, which makes it expensive, impractical and harmful to the environment (WYLIE, 2001; MAHROOF et al., 2002).

Bacillus thuringiensis Berliner is a gram-positive bacterium that produces proteins toxic to various insect orders, including agricultural and forest pests and disease vectors for humans and animals (BAUM et al., 1999). The advantages of using this microorganism are its high specificity in vulnerable insects, its non-polluting effect on the environment, its innocuousness to mammals and vertebrates and the absence of toxicity to plants (WHITELEY; SCHNEPF, 1986). A possible alternative to control *H. grandella* is with the use of biological agents, like fungi (Castro et al., 2017) and *B. thuringiensis* (CASTRO et al., 2018b).

Based on this, this work aims to evaluate the potential for systemic use of the *Bacillus thuringiensis* strains for control of *Hypsipyla grandella* in mahogany seedlings.

2. MATERIALS AND METHODS

This experiment was carried out in a greenhouse at Embrapa Genetic Resources and Biotechnology, Brasília, Federal District. For the production of seedlings, 250 mahogany tree seeds were collected and planted in PlantMax® substrate. After germination, the 200 best seedlings were selected, watered daily and fertilized quarterly with Osmocote® fertilizer. At the end of 12 months, these seedlings were evaluated for height and diameter to select the most homogeneous for the study.

The strains of *B. thuringiensis* used in the experiment were: S1905, S2021, S2122 and S2124 belonging to the Invertebrate Bacteria Collection of Embrapa Genetic Resources and Biotechnology that caused greater than 90% mortality in the selective bioassays (Castro et al., 2018b) and 1450 HD-1 as a positive treatment.

Six seedlings were used for each treatment. Cheesecloth was affixed to each (Silva Junior et al., 2014) containing three eggs of *H. grandella* with 48 hours of age obtained by mass rearing in the Embrapa Insect Rearing Platform (Castro et al., 2016). The eggs were placed close to the terminal bud of the seedling, in order to facilitate the entry of newly hatched caterpillars onto the plant. Therefore, 36 seedlings with 108 eggs of *H. grandella* were used, and the experimental design was completely randomized.

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 hours until complete sporulation when viewed with an optical phase contrast microscope with magnification of 1,000x for spores and crystals. Then the final culture was centrifuged at 9,500 for 30 minutes (Hettich Zentrifugen Centrifuge, model Rotanda 460R), the supernatant was discarded to obtain a concentrated pellet at the end of the work and afterwards it was resuspended in distilled water, frozen and then lyophilized in Christ Lyophilizer, 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) from serial dilutions (MONNERAT et al., 2007). After counting the number of spores, the bacterial lyophilisate was mixed with autoclaved distilled water and the suspension was inoculated via soil in a single dose of 2 mL at a concentration of 1×10^8 spores per mL, for each treatment. The control consisted of autoclaved distilled water without the bacterial lyophilisate.

The assay was evaluated for 30 days after inoculation. During this period, the following parameters were observed daily: gum exudation at the terminal shoot or leaf insertion; presence of *H. grandella* webs; presence of *H. grandella* droppings; behavior and attack of *H. grandella* on seedlings; size of the tunnel formed by *H. grandella*, in centimeters; number of caterpillars per plant; and presence of dead caterpillars inside the plants.

After 30 days, pupae or last instar caterpillars (6th) were observed to be present on each seedling. Parameters related to the development of the insect were observed, such as delay to pupate in relation to the control and assessed to see if inoculation of plants with bacteria could present some harmful effect on the caterpillars. The size of the tunnel formed by *H. grandella* is one of the main parameters for assess the effectiveness of products or the resistance of mahogany to insect attack (OHASHI et al., 2002; SILVA JUNIOR et al., 2014).

The data were submitted to ANOVA variance test and Tukey test ($p<0.05$) using the Assistat statistical program. To evaluate the effectiveness of the bacteria tested, the modified Abbot formula (Abbot, 1925) was used:

$$\text{Effectiveness (\%)} = (\text{CPA-TPA/CPA}) \times 100$$

Where CPA is the number of control plants attacked and TPA is the number of treatment plants attacked. A treatment was considered effective if it had a percent effectiveness of at least 80% and tunnels of less than two centimeters. The rate of mortality of the larvae was also confirmed, since the insect needs to ingest the bacteria for death to occur.

3. RESULTS

All of the larvae hatched two days after the start of the bioassay. They frequently penetrated the axillary bud or terminal shoot first and later moved and fed from non-lignified terminal structures including the axillary bud, new leaves and branches. Feeding continued on the conductive tissue, consuming the phloem and causing destruction of the new shoot and consequent withering of the younger leaves. In isolated cases, attack of the leaf blade was observed. After insect attack of the plants it was possible to observe the presence of gum exudates, and on all occasions the larvae protected their entry and feeding sites by constructing a web, which was covered with plant particles and insect droppings (Figure 1-A). These protective structures were also constructed before pupation, as was also observed by Vergara (1997). Some caterpillars were observed in the central nerve of the leaf (Figure 1-B).

The presence of pupae was not observed until the final experiment, only larvae of various instars. According to Vergara (1997), the climatic conditions and the availability of adequate food directly affected the life cycle, which could be from four to six weeks in duration. During the period in which the experiment was conducted, it was not stated that any plant died.

The plants inoculated with the S1905 strain presented a slower attack compared to control, with few signs of symptoms or damage, characterized by little gummy exudate and minimal presence of droppings and webs from the insect, having an effectiveness of 83.3% and tunnel of 2.20 cm (Table 1). This could indicate a possible delay in the development of the insect or even its death. In addition, four caterpillars were found dead in the interior or near the treated plants, with symptoms of Bt infection (Figure 2).

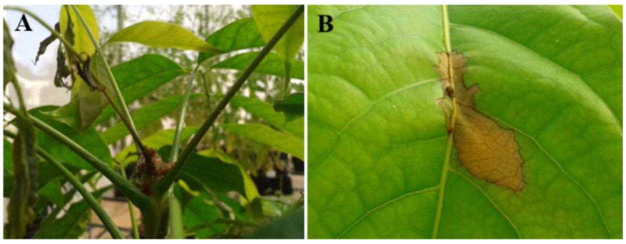

Figure 1 (A-B). Attack of *Hypsipyla grandella* in mahogany seedlings. A- Destruction of the terminal shoot and wilting of new leaves, with the presence of insect droppings and webs; B- Caterpillar on the central nerve of the leaf.
Figura 1 (A-B). Ataque de *Hypsipyla grandella* em mudas de mogno. A- Destruição do broto terminal e murcha de folhas novas, com a presença do excremento do inseto e teia; B- Lagarta na nervura central da folha.

Figure 2. *Hypispyla grandella* caterpillar dead on the mahogany seedling treated with *Bacillus thuringiensis*.
Figura 2. Lagarta morta de *Hypsipyla grandella* em muda de mogno tratada com *Bacillus thuringiensis*.

The S2124 and S2021 strains were similar in effectiveness and tunnel size (Table 1). The S2122 strain presented no effectiveness and the largest sized tunnels, not differing statistically from the plants without Bt, suggesting that the S2122 strain was not able to develop and translocate in the mahogany plants in sufficient quantity to adversely affect the insect (Table 1).

Table 1. Effectiveness of bacterial treatments based on *Hypsipyla grandella* mortality and mean size of the tunnel formed by the insect in mahogany seedlings inoculated with *Bacillus thurigiensis* via soil.
Tabela 1. Eficiência dos tratamentos bacterianos baseados na mortalidade de *Hypsipyla grandella* e no tamanho médio das galerias formadas pelo inseto em mudas de mogno inoculadas com *Bacillus thuringiensis* via solo.

Strain	Effectiveness	Mean size of tunnel (cm)
1450 HD-1	16.7%	8.22 ± 2.29 ab
S1905	83.3%	2.20 ± 0.73 b
S2021	50%	5.83 ± 1.74 b
S2122	0%	15.22 ± 1.24 a
S2124	50%	5.05 ± 2.15 b
Control	0%	13.55 ± 0.96 a

*Means followed by the same letter are not statistically different at 5% significance by Tukey test.

The plants, in all the treatments utilized, presented, to a greater or lesser degree, symptoms typical of attack by *H. grandella*, with construction of webs by the insect, gummy exudate, droppings and the presence of dried leaves. Again the treatment that presented the fewest plants that exhibited these signs was the one with the 1905 strain, followed by the S2021 and S2124, S1450 and S2122 strains and finally the control (Figure 3).

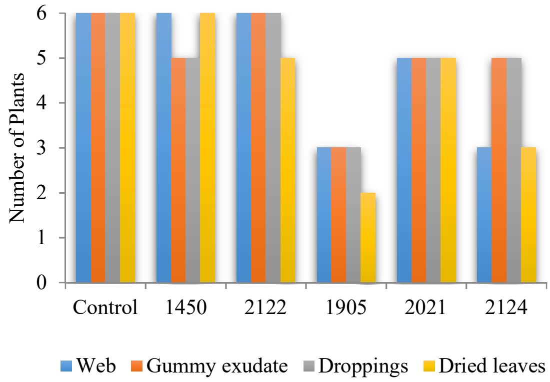

Figure 3. Number of plants presenting webs, gummy exudate, droppings and dried leaves according to the use of different treatments resulting from the attack of *Hypsipyla grandella*.
Figura 3. Número de plantas apresentando teias, exsudação de goma, excrementos e folhas secas de acordo com o uso de diferentes tratamentos quanto ao ataque de *Hypsipyla grandella*.

4. DISCUSSION

The results indicate that some Bt strains used in the experiment possess a systemic effect in mahogany seedlings, with the ability to translocate via conductive vessels. Studies done by Bizarri; Bishop (2007) and Maduell et al. (2007) showed that Bt strains found in the soil and in the phylloplane are the same as the bacteria sometimes transported from the soil to the leaves. Hinton; Bacon (1995) demonstrated that endophytic Bt colonization can begin with the migration of bacteria to the locations where the roots are growing and seeds germinating. Monnerat et al. (2003) demonstrated that Bt colonizes plant tissues of cotton plants and that some strains once inoculated in soils close to the roots of cotton and kale plants spread to all the tissues, reaching the insects (Monnerat et al., 2009), being able to cause their death. Praça et al. (2012) illustrated and confirmed the translocation of Bt in cabbage hybrids, as well as promoting vegetative growth. Coêlho et al. (2011) isolated endophytic bacteria of the genus *Bacillus*, *Pantoea* and two others not cultivated in culture medium in three Amazon tree species. Once in the hostplant, the endophytic microorganism can stay in specific tissues or systemically colonize the plant, establishing a symbiotic, mutualistic, commensal or tropobiotic relationship (ULRICH et al., 2008).

Recent studies have shown that the ability of bacteria to translocate in the plant is determined by the strain and genotype utilized, which indicates that the strain that is effective in the control of certain pest will not necessarily colonize the plant endophytically (HARDOIM et al., 2008; DAVITT et al., 2011). Colonization of mahogany by beneficial microorganisms like Bt can be an important alternative to the use of chemical products for the control of *H. grandella*. Therefore, further investigations and tests with the lyophilisate in trees, especially with strain S1905, should be carried out in order to establish this methodology and formulate a commercial product based on *B. thuringiensis*.

5. CONCLUSIONS

The *Bacillus thuringiensis* strains used in this work possess different toxic effects on *Hypsipyla grandella* when inoculated via soil.

The S1905 strain was demonstrated to be promising for the control of *H. grandella* via systematic effect in mahogany plants, with effectiveness of 83.3%. This work is an early effort in the systemic use of Bt in seedlings of tree species and the method seems to offer a promising and viable alternative to the use of chemical insecticides.

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