THE EFFECT OF *BEAUVERIA BASSIANA* ON BRAZILIAN POPLAR MOTH *CONDYLORRHIZA VESTIGIALIS* (LEPIDOPTERA: CRAMBIDAE)

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Abstract: The Brazilian poplar moth is the most important pest of poplar plantations in Brazil. This research evaluated the effect of *Beauveria bassiana* Bals. (Vuill.) on the mortality and development of *Condylorrhiza vestigialis* Guen. (Lepidoptera: Crambidae). The aim was to develop alternative methods for management of this pest. The pathogens were sprayed on poplar leaves and .consequently, the pathogens reached the caterpillars. *Bacillus thuringiensis* var. *kurstaki* Berliner was sprayed as the standard treatment. The spray for the control was distilled water. Both pathogens *B. bassiana* and *B. thuringiensis* affected insect development with increase mortality at each stage of the insect cycle, reaching a satisfactory control level. Microbial control of Brazilian poplar moth with *B. bassiana* is promising. Tests with other strains and species of pathogens, mainly under field conditions, were also encouraging. This is the first report about the action of *B. bassiana* against *C. vestigialis.*

Key words: *Populus deltoides*, biological control, entomogenous, forestry protection

INTRODUCTION

Poplar plantations (*Populus* spp.) are developing in South America especially in Argentina which has the largest planted area with 65,000 ha, followed by Chile with 6,000 ha (Casaubon *et al*. 2002; Partarrieu 2010). In Brazil, the crops are spreading in the southern part of the state of Parana and the northern part of the state of Santa Catarina, mainly with the species *Populus deltoides* Marsh with approximately 5,000 ha planted. The aim of these plantations is to supply wood for the manufacture of matchsticks, and laminates in general (Otto *et al*. 2007).

Among the factors that affect the Brazilian poplar production are the damage caused by the defoliating caterpillar *Condylorrhiza vestigialis* Guen. (Lepidoptera: Crambidae) known as the Brazilian poplar moth; the most important pest of crop (Marques *et al*. 1995; Diodato and Pedrosa-Macedo 1996). In the larval stage *C. vestigialis* can cause up to 100% defoliation in plantations with two years old, affecting significantly the diameter plant growth in subsequent years. The attacks are primarily from December to March, which coincides with the period of the greatest vegetative growth of plants in the South American continent (Diodato 1999). Actually, the control of this pest is basically done with synthetic insecticides, mainly from the pyrethroids group. However, due to developing resistance in the lowland where poplar is cultivated, it is necessary to search for alternative methods of control. Research must be done to find solutions that are not environmentally harmful. The goal is to decrease

or replace synthetic insecticides. Accordingly, studies with the entomopathogenic virus *Condylorrhiza vestigialis multiple nucleopolyhedrovirus* (CvMNPV) are underway and have shown promising results (Castro *et al*. 2004; Almeida *et al*. 2007; Almeida *et al*. 2008a; Castro *et al*. 2009). Additionally, insecticide based on *Bacillus thuringiensis* is registered for controlling this pest in Brazil. At the same time, there has been little research on entomopathogenic fungi related to controlling *C. vestigialis*. The forestry sector, however, has had several cases of success in the management of other pests with these organisms (Inglis *et al*. 2001; Neuenschwander *et al*. 2003; Almeida *et al*. 2008b). The fungus *Beauveria bassiana* is widely used to control pests and provides a basis for the making of a large number of mycoinsecticides (Inglis *et al*. 2001; Hajek and St. Leger 1994; Wraight *et al*. 2001). Based on such results, this study evaluated the fungus' effect on the mortality and development of *C. vestigialis*, with the aim of developing an alternative method for managing this pest.

MATERIALS AND METHODS

Insects

The experiment was carried out with second instar larvae of *C. vestigialis* obtained from mass rearings maintained by the company Swedish Match do Brasil S.A. For bioassay, the larvae were removed from an artificial diet and separated into plastic cups with four larvae each,

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where they remained without food for 8 h. The cups had a capacity of 80 ml and each cup had a screw cap with a hole approximately 2 mm in diameter to allow aeration. During rearing and bioassay the insects were kept in an incubation chamber; temperature 25±1°C, 75±5% RH, and 12:12 h photoperiod.

Bioassay protocol

Leaves of *P. deltoides* were sprayed with commercial formulations of *B. bassiana*, strain IBCB 66 (Toyobo do Brasil – concentration $1.0x10^{10}$ viable conidia/g) at dosages of 20, 50, 100 and 200 grams of the commercial product c.p./ha in a spray tower. As the standard treatment, leaves were sprayed with *B. thuringiensis* (Dipel WP, Sumitomo Chemical do Brasil) at a dosage of 500 g c.p./ha. For the control, distilled water was used. The spray volume of commercial formulations was equivalent to 150 l/ha + Tween 20 (0.02%). For each treatment, ten poplar leaves obtained from seedlings grown in a vase were chosen. After spraying, leaves were placed to dry naturally and then given to the insects in the cups; there was a total of ten (replicates) per treatment. The insects were exposed to leaves for 48 h. Later, the treated leaves were discarded and replaced by fresh leaves without treatment.

The mortality of *C. vestigialis* was recorded seven days after spraying (DAS), on larvae that did not transformed in pupa, on the pupae, and on the total mortality (accumulated during all stages). Surviving worms transformed into pupa, were placed individually in petri dishes (30 mm diameter) until moth emergence. This procedure was done to observe the effect of insecticides on this stage. The dead insects from the *B. bassiana* treatment, were removed to a moist chamber for verification of pathogen growth.

Data analysis

Mortality rates were transformed in arcsine square-root and submitted to analysis of variance (ANOVA) with means of treatments compared by Tukey's HSD test ($p < 0.05$). The duration of the pupal stage was analyzed by polynomial regression ($p < 0.05$) because emergence of moths only occurred on treatments with *B. bassiana*. For regression analysis, the average of treatments, including the control, were submitted to ANOVA.

RESULTS AND DISCUSSION

The fungus *B. bassiana* was pathogenic to poplar moth. The fungus developed on the host making it possible to watch the pathogen growth; the symptom known as white muscardine (Zimmermann 2007). Observation of the growth was very interesting for those in integrated pest management. The sporulation of the pathogen on the host contributes to the maintenance of inoculum and spread of the disease, optimizing control. Also, the sporulation of entomopathogenic fungi and epizootic potential are desirable characteristics for microbial products (Charnley 1997). Larvae killed by *B. thuringiensis* action showed the characteristic symptoms of this disease, such as flaccid body and blackened tegument (Polanczky *et al.* 2008). No mortality was recorded in the control treatment.

At 7 DAS, *B. thuringiensis* caused the highest rate of larval mortality (56.9%). *B. bassiana* showed low larval mortality with only 26.3%, at a dosage of 200 g c.p./ha and this dosage did not differ from the other doses of fungus and the control (Table 1). Besides the low initial mortality, the majority of the surviving larvae treated with *B. bassiana* that could turn into pupae, also did not differ from control treatment, where all the insects pupate. However, the fungus affected the emergence of moths causing the death of 42.7% pupae and cumulative mortality of 64.6% at the highest dose, reaching a satisfactory level. Similarly, *B. thuringiensis* undertook further development killing all insects during the pupal stage. It is understood therefore, that the pathogens (especially *B. bassiana*) were less lethal to larvae of *C. vestigialis*, but undertook insect biology probably due to disturbances in the feeding process.

Table 1.   Mortality caused by *B. bassiana* and *B. thuringiensis* on *C. vestigialis* in different development stages

Pathogen	Doses [g c.p./ha]	Mortality [%]			
		larvae (7 DAS)	larvae did not pupate	pupa	total
B. bassiana	20	17.5 a	25.2 a	27.7 b	35.2 b
B. bassiana	50	13.1 a	14.6 a	17.7 b	30.0 b
B. bassiana	100	4.9 a	5.1 a	25.0 b	30.4 b
B. bassiana	200	26.3 ab	30.4ab	42.7 b	64.6 bc
B. thuringiensis	500	56.9 b	69.8 b	100.0 c	100.0 c
Control	–	0.0 a	0.0 a	0.0 a	0.0 a
F		5.77*	5.15*	16.98*	13.61*
p-valor		0.0019	0.0035	<0.0000	<0.0000

Means followed by the same letters in the same column do not differ according to the Tukey HSD test ($p < 0.05$);
DAS – days after spraying

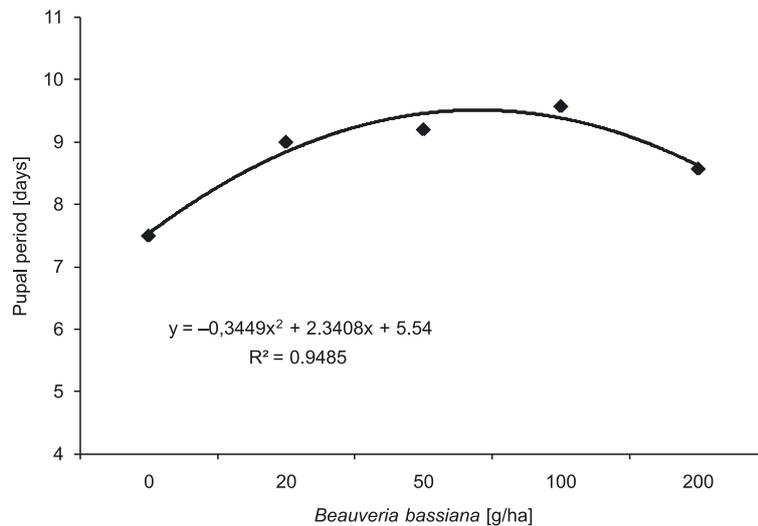$$y = -0{,}3449x^2 + 2.3408x + 5.54$$
$$R^2 = 0.9485$$

Fig. 1. Duration of pupal stage of *C. vestigialis* survivors to treatment with *B. bassiana* ($p < 0.05$)

This is because these disorders are reflex symptoms of expression mechanisms of disease which affects the absorption of nutrients from the host, resulting on energy loss (Nolan and Clovis 1985). As the larval stage is intended solely for accumulation of food reserves, any disturbance at this stage may reflect negatively on development, reproduction, fertility and survival of insects (Fargues *et al*. 1991; Costa and Ide 2006). This negative effect was observed on *Helicoverpa zea* Bod. and *Heliothis virescens* Fabr. (Lepidoptera: Noctuidae) exposed to *B. thuringiensis* and on *Otiorhynchus sulcatus* Fabr. (Coleoptera: Curculionidae) when exposed to *B. brongniartii* (Sac.) Petch (Abbas and Young 1993; Kowalska 2008). In this work, in addition to mortality, *B. bassiana* affected the development of the insect in the longer lasting pupal stage (Fig. 1). Observation was not possible with *B. thuringiensis* due to the death of all pupae. In others experiments the longest duration of the pupal stage was also observed in *Musca domestica* L. (Diptera: Muscidae) infected with *Brevibacillus laterosporus* Laub. (Ruiu *et al*. 2006) and *Choristoneura fumiferana* Clem. (Lepidoptera: Tortricidae) infected with *B. bassiana* (Bauer and Nordin 1988a). The stretching of some stages of development can be beneficial by increasing exposure of insects to natural enemies and contributing to pest population decrease.

With the exception of *B. thuringiensis*, the way that insects were exposed to *B. bassiana* (residual) may have contributed to the lower larvae susceptibility. The greatest effect of the pathogen *B. bassiana* is achieved by contact through tegument, in contrast *B. thuringiensis* acts exclusively by ingestion (Alves 1998). Probably a lethal dose of fungus could not be acquired efficiently by larvae, or this stage was not susceptible to the pathogen , though this stage is susceptible in other insects (Veerstergaard *et al*. 1995). The occurrence of ecdysis after inoculation may also influence the effectiveness of pathogens (Butt and Goettel 2000). However, in the field, the spraying plants directly, affects the insect, or the insects become infected by eating or walking on the residue from the surface, varying the amount of infective propagules acquired by the host (Goettel *et al*. 2000). This variation can result in

a lethal or a sublethal dose, causing death or consequences on insect development, respectively (Royama 1984; Bauer and Nordin 1988b; Giustolin *et al*. 2001; Hornbostel *et al*. 2004). Moreover, according to our results the assessment of mortality without considering the whole cycle of the host, may underestimate the control potential of some entomopathogenic fungi. It is necessary to observe secondary infection effects. Under field conditions, these effects can significantly reduce the feeding of insects. The result is lower leaf consumption and reduction of economic losses in production, in addition to the reduction of the pest population caused by reduced fertility of individuals (Goettel *et al*. 2000).

The microbial control of *C. vestigialis* with *B. thuringiensis* was highly efficient while the control with the entomopathogenic fungus *B. bassiana* is promising, encouraging studies with others strains and species of entomopathogenic fungi. Studies must mainly be done on the effect of these agents on bioecological parameters of insect under field conditions. This is the first report about the action of *B. bassiana* against *C. vestigialis*.

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