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Effect of *Bt* Soybean on Larvae of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)

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Abstract

Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae) causes economically significant damage to soybeans *Glycine max* (L.) Merrill (Fabaceae: Phaseoleae). The genetically modified soybean expressing the Cry1Ac protein of *Bacillus thuringiensis* Berliner, which is toxic to lepidopterans, is a potential alternative tool to manage this pest. Bioassays with *H. armigera* larval instars were conducted with *Bt* (Cry1Ac) and non-*Bt* soybean plants in order to evaluate the efficacy of control with the modified cultivar. *Bt* soybean affected the mean time of mortality in each larval instar, but its efficacy was not affected by the larval stage. Mortality was 100% in all six instars, indicating that the *Bt* soybean expressing the Cry 1Ac protein is an efficient control tactic for this pest.

Keywords: *Bacillus thuringiensis*, control, Cry1Ac, Heliothinae, instar, MON 87701

1. Introduction

The larvae of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) are known for their high population outbreaks, difficulty of control, and enormous damage to crops worldwide (Alvi et al., 2012; Degrande & Omoto, 2013; Feng et al., 2010). The larvae are polyphagous and damage many commercially important crop plants including cotton, legumes in general, sorghum, corn, and tomato, among others (Liu et al., 2010; Chelliah et al., 2011).

Helicoverpa armigera is among the most important agricultural pests in Asia, Africa, Europe, and Australia (Tay et al., 2013). Recently it was reported in the Americas, including several Brazilian states and in several crops including the soybean *Glycine max* (L.) Merrill (Fabaceae: Phaseoleae) (Czepak et al., 2013a; Specht et al., 2013). This pest species is highly adaptable, with a high reproduction rate, wide distribution, and capability to feed and develop on different host species, which results in significant economic losses due to the difficulty of control (Cpezak et al., 2013b).

In soybeans, *H. armigera* is highly destructive to the plant vegetative and reproductive structures, requiring control in order to prevent losses in production. Traditionally, the species has been managed mainly through chemical control; however, it rapidly develops resistance to pesticides (Bués et al., 2005; Alvi et al., 2012). *Bt* soybean, which expresses the Cry1Ac protein from *Bacillus thuringiensis* Berliner, is a potential alternative control measure for *H. armigera* populations, since it can be used in managing the species (Yu et al., 2013). On the other hand, *H. armigera* is not listed as a target pest for this technology in Brazil, since the pest was introduced into the country only after the Brazilian government agencies had authorized the commercial sales of first generation of *Bt* soybean (Intacta©). Because cultivation of *Bt* soybean in Brazil is quite recent, few studies have examined the effects on *H. armigera*; and to our knowledge, no published articles have reported on the mortality of different instars of this pest exposed to this cultivar. The efficacy of control technologies derived from *B. thuringiensis* may differ during the larval development of lepidopterans (Rausell et al., 2000; González-Cabrera et al., 2011). González-Cabrera et al. (2011), Aggarwal et al. (2006) and Rausell et al. (2000) reported that advanced instars of insects are usually more tolerant to biological pesticides and purified *Bt*

proteins. Bernardi et al. (2012) reported that advanced instars are presumably more tolerant to *Bt* proteins, and thus larval mortality in these instars indicates that a species is highly susceptible to these toxins. In general, the more advanced the instar, the greater the difficulty of controlling it, for the larvae must ingest a larger amount of tissue to cause lethality, with consequent damage to the plant.

In addition, the sequential availability of alternative hosts in the field can support the larvae and allow different instars to attack *Bt* soybean plants, causing a certain degree of damage. For instance, it is common to find *H. armigera* larvae in the stubble and weed plants that precede soybean planting, which function as a "green bridge" between crops; after burndown they begin to feed on the legumes, starting the first generation of the field pest early in the season (Carvalho et al., 2014).

In practice, it is necessary to prevent the older larvae from accessing seedlings, in order to minimize selection pressure and possible sub lethal exposure to the *Bt* protein. For this reason, a prior burndown with insecticide is recommended as good agricultural practice. However, if *Bt* soybean is able to control all larval instars, insecticides will not be required during the burndown period.

The present study evaluated the control efficiency of the *Bt* soybean, which expresses the Cry1Ac protein, on all larval instars of *H. armigera*.

2. Material and Methods

The bioassays were performed in the Laboratório de Entomologia Aplicada (FCA, Faculdade de Ciências Agrárias) of the Universidade Federal da Grande Dourados (UFGD), in the city of Dourados, state of Mato Grosso do Sul (MS), Brazil.

The rearing colony of *H. armigera* was established from larvae collected in soybean fields in São Gabriel D'Oeste, MS, in November 2014. The larvae were placed in 100 mL plastic containers covered with absorbent paper and were fed daily with non-*Bt* soybean leaves until they pupated. The pupae were removed from the containers and stored in Petri dishes containing vermiculite. After emerging, the adults were kept in a climate-controlled room [25 ± 2ºC, relative air humidity (RH) of 70 ± 10% and photophase of 12 h], in PVC tubes (15 cm diameter and 22 cm high) covered on the bottom with a Petri dish, and on the top with tulle mesh. The inner surface of each tube was covered with sheets of white paper that functioned as an oviposition substrate. The adults were fed by means of cotton balls soaked in a 10% honey solution and placed on the tulle mesh. The eggs laid on the paper and the mesh were transferred to plastic bags (2 Kg capacity) previously moistened with distilled water. The food was exchanged and the feces removed daily.

After they hatched, the larvae to be used in the bioassays were fed with soybean leaves of the non-*Bt* variety BMX Potência until they reached the instar to be evaluated. A parallel study was conducted to determine the instars of *H. armigera* on the soybean.

The second generation of the insects obtained in the laboratory was used in the study. For each larval instar, the mortality was evaluated in separate experiments. Both soybean varieties, *Bt* Monsoy 5947 I PRO, which expresses the Cry1Ac protein, and non-*Bt* Nidera 5909 RR were grown in a greenhouse in plastic pots with a 10-liter capacity, containing a mixture of soil, sand, and organic substrate (1:1:1). After the plants sprouted, they were thinned to five plants per pot and received appropriate care and irrigation.

Fully expanded leaves were removed from the middle part of the plants after they reached phenological stages R1-R2 (Fehr & Caviness, 1977) for the evaluation of the 1st-instar larvae, stages R2-R3 for the evaluation of the 2nd- and 3rd-instar larvae, R3 for the evaluation of the 4th-instar larvae, and R4 for the evaluation of the 5th and 6th instars. The leaves were placed in plastic containers (150 ml) containing moistened filter paper. A larva of the instar to be evaluated was placed on each leaf. The leaves were exchanged every two days. The containers were checked for dead larvae every 24 hours, until all larvae had died.

The experimental design of each bioassay consisted of two treatments and 40 repetitions (individual larvae). The survival data were analyzed with the Kaplan-Meier (Gehan-Breslow) test.

3. Results

Neonate larvae of *H. armigera* were susceptible to the Cry1Ac protein expressed in the *Bt* soybean leaves, which caused 100% mortality four days after the infestation, significantly higher than in the non-*Bt* variety (86% survival) (K-M = 37.627, d.f. = 1, p = 0.001) (Figure 1A). The larvae caused little damage to the *Bt* soybean leaves, only scraping a small amount of tissue, which was enough to kill the 1st instar.

The survival of the 2nd instars was also lower in *Bt* soybean compared to non-*Bt* (K-M = 51.671, d.f. = 1, p = 0.001) (Figure 1B).

For the 3rd instars, neither cultivar of *Bt* and non-*Bt* soybean caused mortality of *H. armigera* larvae in the first 24 h after the infestation (Figure 1C). On the *Bt* variety, 50% mortality of 3rd instars occurred two days after the infestation, and at this time the percentage of mortality was highest. After five days of infestation, all the larvae fed with *Bt* soybean leaves had died, while 95% of the control larvae (fed with non-*Bt* soybean) survived (K-M = 75.163, d.f. = 1, p = 0.001).

In 4th-instar larvae, *Bt* soybean caused 100% mortality at a mean of five days after the infestation, showing a significant difference from larvae on the non-*Bt* soybean (K-M = 57.660, d.f. = 1, p = 0.001) (Figure 1D).

For the 5th- and 6th-instar larvae, 100% mortality for the *Bt* soybean was reached after four days, differing significantly from the larvae on the non-*Bt* soybean, where 91% of 5th-instar larvae (K-M = 43.976, d.f. = 1, p = 0.001) and 70% of 6th-instar larvae (K-M = 31.464, d.f. = 1, p = 0.001) survived (Figures 1E and 1F).

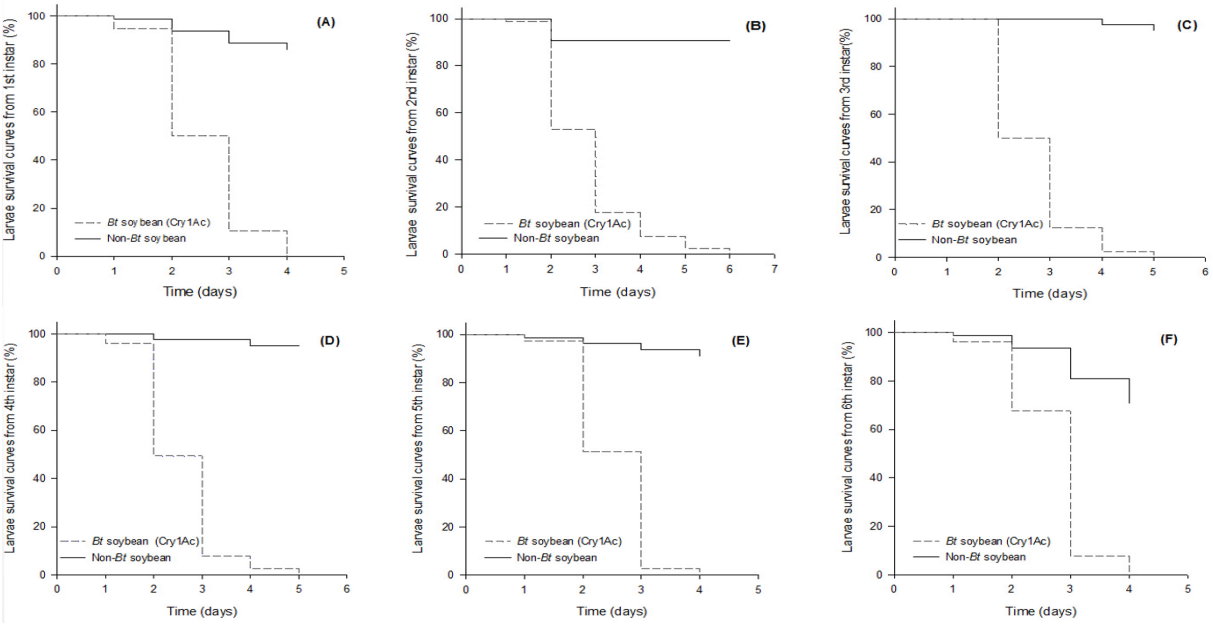

Figure 1. Larvae survival curves from 1st (A), 2nd (B), 3rd (C), 4th (D), 5th (E) and 6th instars of *Helicoverpa armigera* fed with *Bt* (Cry 1Ac) and non-*Bt* soybean plants

4. Discussion

Bt soybean affected the mean time of mortality for *H. armigera* during development, but the overall efficacy of this cultivar was not affected by the age of the larvae, since all instars eventually died. Yu et al. (2013) found in bioassays that soybean plants that express the Cry1Ac protein are highly resistant to *H. armigera* because of the insect's sensitivity to this toxic protein. Bernardi et al. (2014) also observed complete mortality in 1st- to 5th-instar larvae of *Heliothis virescens* after they were exposed to the *Bt* soybean, showing the efficacy of this technology for control of lepidopterans feeding on this soybean cultivar.

For neonate larvae, 100% mortality, which in this study occurred four days after the infestation, was also observed in *Anticarsia gemmatalis* and *Chrysodeixis includens*, in bioassays with leaf disks of *Bt* soybean MON 87701 × MON 89788 (Bernardi et al., 2012).

The bioassays by Yu et al. (2013) also showed that the *Bt* soybean that expresses the Cry1Ac protein is highly resistant to 2nd-instar larvae of *H. armigera* during the different periods of soybean plant growth. Yu and coworkers evaluated the larval survival for a period of four days of feeding, and at the end of their observations, they observed that the surviving larvae fed on *Bt* soybean weighed less than larvae fed with non-*Bt* soybean. This lower weight could affect the survival of the larvae and lead to the death of all the subjects at later stages; this pattern agrees with the results presented here, where 100% larval mortality was observed six days after the infestation in *Bt* soybean.

Bernardi et al. (2012), in studies with *A. gemmatalis* and *C. includens* in greenhouses, also observed that larvae from advanced instars from contour-line non-*Bt* plants when migrating to *Bt* soybean plants were controlled by

MON 87701 × MON 89788 soybean. Bernardi and colleagues also reported that the high mortality of these two lepidopteran species, even in advanced instars, which are presumably more tolerant, is the result of the high expression of the *Bt* protein in MON 87701 × MON 89788 soybean and the susceptibility of these species to Cry1Ac. The present study found similarly high mortality for the different *H. armigera* instars.

The shorter lethal period for the 5th and 6th instars of *H. armigera* might be due to the fact that these instars are larger, consume more, and also are preparing for pupation (when they do not feed); the higher food intake results in ingestion of a larger amount of toxic protein, which may accelerate mortality.

Our results suggest the desirability of future studies to assess the efficacy of the reproductive structures of this soybean cultivar, to determine if the different plant structures might vary in their efficiency in controlling the different instars of *H. armigera*. Alternatively, the level of expression of Cry 1Ac might vary during the growth of the plants (Yu et al., 2013) and/or in the different plant structures, as is the case for the *Bt* cotton plant (Arshad et al., 2009). Selection for resistant subjects may also occur in the field, accelerating the evolution of resistance of the pest; for instance, Gunning et al. (2005) reported that an Australian population of *H. armigera* has shown resistance to the Cry1Ac toxin expressed in the *Bt* cotton plant.

Because this species feeds on several crops (Gunning et al., 2005), there is some risk that populations in Brazil might develop resistance to Cry 1Ac because the protein is present in both *Bt* soybean and *Bt* cotton, and successive planting of these cultivars might extend the period of pest exposure to the protein and hence increase the possibility of selecting resistant individuals (Bernardi et al., 2014). Therefore, a high degree of control of *H. armigera* in *Bt* soybean and consequent reduction of damage from this pest will be necessary to maintain the effective refuge areas, aiming toward the sustainability of the technology.

Our results indicate that the *Bt* soybean that expresses the Cry 1Ac protein provides efficient control of all six larval instars of *H. armigera*.

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