Revista Brasileira de Entomologia 59 (2015) 7-11

REVISTA BRASILEIRA DE
Entomologia
A Journal on Insect Diversity and Evolution
www.sbe.ufpr.br/

Biological Control and Crop Protection

Larval development of *Spodoptera eridania* (Cramer) fed on leaves of *Bt* maize expressing Cry1F and Cry1F + Cry1A.105 + Cry2Ab2 proteins and its non-*Bt* isoline

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ARTICLE INFO

Article history:
Received 8 May 2014
Accepted 1 December 2014
Associate Editor: Daniel R. Sosa-Gómez

Keywords:
Lepidopteran pest
Southwest armyworm
Transgenic corn
Non-target pest

ABSTRACT

This study aimed to evaluate, in controlled laboratory conditions (temperature of 25±2 °C, relative humidity of 60±10%, and 14/10 h L/D photoperiod), the larval development of *Spodoptera eridania* (Cramer, 1784) (Lepidoptera, Noctuidae) fed with leaves of *Bt* maize expressing Cry1F and Cry1F + Cry1A.105 + Cry2Ab2 insecticide proteins and its non-*Bt* isoline. Maize leaves triggered 100% of mortality on *S. eridania* larvae independently of being *Bt* or non-*Bt* plants. However, it was observed that in overall *Bt* maize (expressing a single or pyramided protein) slightly affects the larval development of *S. eridania*, even under reduced leaf consumption. Therefore, these results showed that Cry1F and Cry1F + Cry1A.105 + Cry2Ab2 can affect the larval development of *S. eridania*, although it is not a target pest of this plant; however, more research is needed to better understand this evidence. Finally, this study confirms that non-*Bt* maize leaves are unsuitable food source to *S. eridania* larvae, suggesting that they are not a potential pest in maize fields.

Introduction

Among the biological pest control technologies currently available in the market, the cropping of genetically modified plants, mainly expressing genes of *Bacillus thuringiensis* Berliner (*Bt*), has grown exponentially worldwide (James, 2013). The increasing use of OGMs is due to the reduced use of insecticides that this technology provides for pest management programs, consequently reducing costs for producers (Qaim and Mathuschke, 2005; Werle et al., 2011). However, this change of pest control strategy may favor the incidence of other non-target insects that are currently of secondary importance to non-*Bt* crops. Some changes in the population dynamics of insect-pests were already reported in different countries of Europe and South America, where *Bt*-maize favored the population growth of non-target pests such as aphids and leafhoppers, although the reason for these changes is not still very clear (Faria et al., 2007; Virla et al., 2010).

The non-target pests are those insects that through ingestion of *Bt*-plants are exposed to Cry toxic proteins for a long time period, but are not direct targets of such technology (Andow and Hilbeck, 2004). The farming areas grown with *Bt*-plants may favor the population increase of non-target pests for two main reasons: (1) reduction on use of broad spectrum insecticides, which increases mortality of the secondary pests (Lu et al., 2010), and (2) lower competition for food, what consequently may cause an increase in population of these non-target pests within the cropped area (Zeilinger et al., 2011). The caterpillar *Spodoptera eridania* (Cramer, 1784) (Lepidoptera, Noctuidae) is one of the lepidopteran species of secondary importance that are reported to occur in areas cultivated with maize (Manuwoto and Scriber, 1985). Such insect species is highly polyphagous and its host range includes horticultural plants (Michereff-Filho et al., 2008), fruiting plants (Bortoli et al., 2012; Zenker et al., 2010), and ornamental plants (Delaney, 2012). Its economic importance seems to be increasing in major annual crops, such as soybean and cotton (Bueno et al., 2011; Santos et al., 2005; Santos et al., 2010).

In face of the foregoing, it may be inferred that larvae of *S. eridania* display a wide capacity of adaptability to different agroecosys-

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0085-5626/
http://dx.doi.org/10.1016/j.rbe.2014.12.001

tems, for they may use the leaves of various crops as food source. However, information regarding the impact of *Bt* technology on the development of high infestations of this pest on non-*Bt* maize crops is still scarce. Nevertheless, this information is crucial to the development of adequate planning of integrated pest management for both types of maize, *Bt* genotypes (Cry1F and Cry1F + Cry1A.105 + Cry2Ab2) as well as for its non-*Bt* isolines. Therefore, this study aimed at studying the comparative biology of *S. eridania* fed with leaves originating from two *Bt* maize isolines, expressing Cry1F and Cry1F + Cry1A.105 + Cry2Ab2 proteins, and its near non-*Bt* maize isoline.

Material and methods

Experimental conditions and insect colony

The study was carried out in the entomology laboratory of Embrapa Soybean, and the experiments were performed into BOD type climatic chambers under controlled environmental conditions of temperature (25 ± 2 °C), relative humidity (60 ± 10%), and photoperiod [14/10 h (L/D)]. Larvae of *S. eridania* used in the study were obtained in the insect mass rearing of Embrapa Soybean, where these insects have been reared for around 20 generations, following the methodology described by Pomari et al. (2012).

Maize plants

The two *Bt* maize isolines evaluated in the study were Herculex® I (Dow AgroSciences) and PowerCore® (Dow AgroSciences and Monsanto), and its near non-*Bt* isoline, the hybrid 2B688 DOW (Dow AgroSciences). Genotype Herculex® I (event TC1507) contains the gene encoding for the insecticidal protein Cry1F, and genotype PowerCore® (event MON89034 x MON00603 x DAS01507) contains five genes with stacking traits that encode for three different *Bt*-proteins (Cry1F + Cry1A.105 + Cry2Ab2) with insecticidal effect, and two genes (PAT + EPSPS) that encode for tolerance to the herbicides glyphosate and glufosinate (Santos et al., 2012).

Sowing of the three maize genotypes was performed into pots, each containing 8 L of sterilized soil as substrate, which were then transferred to a greenhouse. For all genotypes assessed (*Bt* and non-*Bt*) five seeds per pot were used, thereby totaling 75 plants to each genotype. Substrate irrigation was performed before sowing, as well as immediately after sowing, to ensure uniformity of soil moisture within all pots; after seedling emergence, irrigation was performed daily. Chemical fertilization was carried out in post emergence through the application of nitrogen [$(NH_4)_2SO_4$], phosphorus (P_2O_5) and potassium (K_2O), following the technical recommendations indicated to the crop (Fancelli and Dourado-Neto, 2000). Throughout the crop cycle application of herbicides, fungicides or insecticides was not carried out in order to avoid interference of those chemical products on the results obtained.

To feed the larvae, leaves from the three maize genotypes were collected daily, starting when plants reached the vegetative growth stage V_8 (eight fully developed leaves). Soon after collection, leaves were cut into pieces (about 30 cm^2 each), and before being offered to the larvae the leaf sections were disinfected by immersion in a 5% sodium hypochlorite solution for 15 minutes. After such period, sections were removed from the solution and left to dry for 30 minutes, for solution evaporation.

Biological characteristics of S. eridania *larvae when fed on Bt and non-Bt maize at the first and the third instar*

Two independent bioassays were carried out. In the first bioassay, newly hatched larvae (larvae up to 24 hours old) were individualized with the aid of a thin tip brush (0.6 mm) into paraffined cups with a 50 mL capacity, and fed according to the previously described treatments. Differently for the third-instar bioassay, the newly hatched larvae were fed an artificial diet (Kasten et al., 1978) until reaching the third instar, when they were then transferred to the respective treatments.

In both bioassays, larvae of each replicate were fed leaf sections previously prepared (treatment) and offered *ad libitum*. A cotton pad soaked in sterile water was placed at the base of each leaf section to slow the drying of leaves. Leaf sections were replaced daily at the same time in which the larval instar and the mortality rate and development (days) of larvae were daily assessed. The two bioassays were carried out in a completely randomized, experimental design, with three treatments [two *Bt* isolines (expressing Cry1F and Cry1F + Cry1A.105 + Cry2Ab2) and its near non-*Bt* isoline (genotype 2B688 DOW)], and 10 replications. Each replicate was composed of eight larvae individualized into paraffined cups, thereby totaling 80 larvae to each treatment.

Biological characteristics and foliar consumption of fifth instar S. eridania *larvae fed Bt and non-Bt maize*

This bioassay was carried out following the same methodology previously described for both the first and the third instars bioassays. However, since these previous bioassays resulted in a 100% *S. eridania* mortality, in this essay the larvae were reared on an artificial diet until reaching the fifth instar, when they were then transferred to paraffined cups, and *Bt* and non-*Bt* maize leaves were offered *ad libitum* to larvae, and leaf consumption by the *S. eridania* larvae was assessed.

According to Bueno et al. (2011) over 90% of larvae consumption occurs at the 5th and the 6th instar, which indicates that this methodology is valid to compare this parameter between treatments. To assess foliar area reduction caused by the dehydration, the foliar sections of the maize leaves that remained in the paraffined cups without larva were measured and used as control, thereby allowing correction of the total leaf area consumed by the larvae. Assessments of leaf area consumed by the larvae were performed daily with the aid of a foliar area meter (brand LI-Cor, model LI-Cor AM 300), until the interruption of their feeding habits.

Statistical analysis

Results of the different bioassays were subjected to exploratory analyzes to assess the assumptions of normality of residuals (Shapiro and Wilk, 1965), the homogeneity of variance of treatments, and additivity of model (Burr and Foster, 1972), to allow for ANOVA application. The means were compared by Tukey test (SAS Institute Inc., 2001), and difference was considered significant only when the significance level was $P \leq 0.05$.

Results

Biological characteristics of S. eridania *larvae when fed on Bt and non-Bt maize at the first and the third instar*

Newly hatched *S. eridania* larvae (first instar) fed on leaves of both *Bt* isolines (expressing Cry1F and Cry1F + Cry1A.105 + Cry2Ab2 proteins) showed a slightly shorter development period (1 day) than larvae fed on leaves of the non-*Bt* genotype (1.7 days) (Table 1). Nevertheless, *Bt* and non-*Bt* maize leaves triggered 100% mortality during the first instar on *S. eridania* larvae (Table 2).

The second bioassay again showed a slight impact of *Bt* plants, and a higher mortality before larvae molting until fourth instar on *Bt* maize expressing Cry1F + Cry1A.105 + Cry2Ab2 proteins (Table 2). Among the surviving larvae that reached the fourth instar, the mortality rate was lower on the non-*Bt* maize, but it reached 73% (Table 2).