

Larval development of *Spodoptera eridania* and *Spodoptera frugiperda* fed on fresh ear of field corn expressing the *Bt* proteins (Cry1F and Cry1F + Cry1A.105 + Cry2Ab2)

Desenvolvimento larval de *Spodoptera eridania* e *Spodoptera frugiperda* alimentadas com espigas de milho verde expressando proteínas *Bt* (Cry1F e Cry1F + Cry1A.105 + Cry2Ab2)

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— NOTE —

ABSTRACT

The objective of this study was to evaluate extent of larval period, larval survival (%), food consumption, and pupal biomass of *Spodoptera eridania* and *Spodoptera frugiperda* (Lepidoptera: Noctuidae) fed on fresh ears of field corn expressing *Bt* proteins (Cry1F and Cry1F+Cry1A.105+Cry2Ab2). Larvae of *Spodoptera* spp. survived less than two days when they consumed *Bt* corn cobs and showed 100% mortality. *Spodoptera eridania* reared on non-*Bt* corn cobs showed higher larval development (21.6 days) than *S. frugiperda* (18.4 days) and lower viability (56.4% and 80.2% for *S. eridania* and *S. frugiperda*, respectively). A higher amount of corn grains was consumed by *S. eridania* (5.4g) than by *S. frugiperda* (3.9g). In summary, this study demonstrated that the toxins Cry1F and Cry1F + Cry1A.105 + Cry2Ab2 expressed in fresh corn cobs contributed to protect ears of corn against *S. frugiperda* and the non-target pest *S. eridania*. However, it is important to monitor non-*Bt* cornfields because of the potential of both species to cause damage to ear of corn.

Key words: biology, integrated pest management, fall armyworm, Southern armyworm, varietal resistance.

RESUMO

Este estudo teve como objetivo avaliar o período de desenvolvimento larval, sobrevivência larval (%), consumo de alimento e biomassa pupal de *Spodoptera eridania* e *Spodoptera frugiperda* (Lepidoptera: Noctuidae), alimentadas com grãos verdes de milho *Bt* que expressam as proteínas Cry1F e Cry1F + Cry1A.105 + Cry2Ab2). As larvas de *Spodoptera* spp. alimentadas com milho *Bt* sobreviveram por até dois dias, registrando-se 100% de mortalidade. A espécie *S. eridania* alimentada com grãos de milho não *Bt* apresentou um maior desenvolvimento larval (21,6 dias) do que *S. frugiperda* (18,4 dias), e menor viabilidade larval

(56,4% e 80,2% para *S. eridania* e *S. frugiperda* respectivamente). Uma maior taxa de consumo de grãos foi observada para *S. eridania* (5,4g) do que para *S. frugiperda* (3,9g). Em síntese, este estudo comprova que as toxinas Cry1F e Cry1F + Cry1A.105 + Cry2Ab2 expressas em grãos imaturos de milho contribuem na proteção das espigas de milho contra *S. frugiperda* e a praga não-alvo *S. eridania*, entretanto, é importante monitorar as lavouras cultivadas com milho não *Bt*, devido o potencial de ambas as espécies em ocasionar danos nas espigas da planta.

Palavras-chave: biologia, manejo integrado de pragas, lagarta-do-cartucho, lagarta-das-vagens, resistência varietal.

For integrated pest management (IPM), the use of resistant genetically modified organisms (GMOs) contributes in reducing the insecticides applications and environmental impact. Cultivation of improved plants that express the insecticidal gene *cry* via the bacterium *Bacillus thuringiensis* Berliner (*Bt*) is performed almost worldwide (JAMES, 2013). Efficiency of the Cry1F protein for controlling *Spodoptera frugiperda* (Lepidoptera: Noctuidae) has been reported (BUNTIN, 2008; SIEBERT et al., 2008), but it was observed when only maize leaves were offered. Recently, BORTOLOTTO et al. (2015) reported the slightly poor development of *Spodoptera eridania* (Lepidoptera: Noctuidae) when fed with maize leaves that expressed single Cry1F and pyramided

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Cry1F + Cry1A.105 + Cry2Ab2 insecticidal proteins; however, the impact of these *Bt* proteins remains unclear because of high larval mortality even when non-*Bt* maize leaves were offered.

Spodoptera spp. can damage the reproductive structures of plants (KING & SAUNDERS, 1984), and there is little information on the development of the *Spodoptera* complex in corn cobs; studies are required to formulate implementation strategies for IPM. In this study, we aimed to assess the impact of the *Bt* protein Cry1F and pyramided protein Cry1F + Cry1A.105 + Cry2Ab2 expressed in fresh corn cobs on the larval development of *S. eridania* and *S. frugiperda*.

The study was performed in the entomology laboratory of Embrapa Soybean, and the experiments were performed using BOD type climatic chambers under the following controlled environmental conditions: temperature, 25±2°C; relative humidity, 60±10%; and photoperiod, 14/10h (L/D). Larvae of *S. eridania* and *S. frugiperda* were obtained by mass insect rearing at Embrapa Soybean, where the insects were reared for about 20 generations.

The two *Bt* corn isolines used in the study were as follows: Herculex® I (event TC1507), which contained the gene for the insecticidal protein Cry1F, and genotype PowerCore® (event MON89034 x MON00603 x DAS01507), which contained five genes with stacking traits that code for three different *Bt* proteins (Cry1F + Cry1A.105 + Cry2Ab2) with insecticidal effects and two genes (PAT + EPSPS) that code for tolerance to the herbicides glyphosate and glufosinate. The non-*Bt* isoline used was genotype 2B688 DOW. Sowing of corn genotypes was performed in a greenhouse. After seedling emergence, irrigation was performed daily. Chemical fertilization was performed according to the regional recommendation (FANCELLI & DOURADONETO, 2000). Corn cobs were harvested in the dough stage (paste-like) and immediately peeled to feed *S. eridania* and *S. frugiperda* larvae.

Comparison of the biological aspects of *S. eridania* and *S. frugiperda* larvae was carried out using a completely randomized experimental design, with three treatments (two isogenic *Bt* corn genotypes and one non-*Bt* corn genotype) and 11 replications. Each replicate was composed of eight larvae. Newly hatched larvae (until 24 hours of age) were individually added using a thin-tip brush (0.6mm) into paraffin cups (capacity, 50ml) for each treatment, resulting in a total of 88 larvae for each treatment. Larvae for each replicate were fed with pieces of fresh corn cobs prepared previously (treatments).

Fresh corn cobs were husked and sliced into pieces for the larvae. The food was replaced daily at the same time, and larval instar and larvae mortality rate were assessed daily until the larvae reached the pupal stage.

When the larvae reached the fifth instar stage, consumption of corn grains was assessed by the weight difference of corn cobs sliced before feeding and the weight of the same corn cobs 24h after feeding. Pieces of corn cobs were weighed before they were offered to the fifth instar larvae to verify the consumption rate, and the weight of food consumed was corrected with the value obtained for the weight of corn grains used in the control treatment (without larva) to assess the weight lost by the grains due to dehydration. Larvae were fed with fresh corn grains *ad libitum* until they completed the larval phase, i.e., until the pre-pupal phase. Results obtained for *S. eridania* larvae were then compared with those obtained for *S. frugiperda* larvae. Within 24h after the larvae reached the pupal stage, the individuals were weighed using an analytical balance with 0.001g of precision. Parameters assessed for both species were as follows: extent of the larval period (days); larval survival (%); grain consumption (g); and pupal biomass (g).

Results of the different bioassays were subjected to exploratory analysis to assess the assumptions of normality of residuals, homogeneity of variance of treatment, and additivity of the model to allow for application of analysis of variance. Mean values were compared using Tukey's test. To compare biological parameters and grain consumption, Student's *t*-test was used. For both tests, the difference was considered significant only when the significance level was equal to $P \leq 0.05$.

This study demonstrated 100% mortality for *S. eridania* and *S. frugiperda* fed on fresh grains to both *Bt* events with expression of a single (Cry1F) and pyramided event (Cry1F + Cry1A.105 + Cry2Ab2) (Table 1). Thus, although *S. eridania* is a non-target pest, these proteins act against its development. When larvae were fed with fresh grains of the non-*Bt* corn genotype, a large percentage of the larvae were able to complete larval stage; this indicates that the grains are nutritionally suitable for the development of *S. eridania* larvae. This is an important observation because *S. eridania* larvae has a poor development when fed on maize leaves (BORTOLOTTO et al., 2015). Higher corn cob consumption by *S. eridania* rather than by *S. frugiperda* was observed, but lower pupal biomass was reported for *S. eridania*. Nevertheless, the pupal biomass of *S. eridania* collected in the present

Table 1 - Biological aspects of *Spodoptera eridania* and *Spodoptera frugiperda* (Lepidoptera: *Noctuidae*) fed with *Bt* and non-*Bt* fresh corn cobs.

-----Biological aspects-----				
Treatment	Larval period (days) ¹	Survival (%) ¹	Consumption of corn cobs (g) ²	Biomass pupal (g) ²
<i>S. eridania</i> on non- <i>Bt</i> isogenic (DOW 2B688)	21.6 ± 0.7 a	56.4 ± 6.8 b	5.4 ± 0.3 a	0.26 ± 0.1 b
<i>S. frugiperda</i> on non- <i>Bt</i> isogenic (DOW 2B688)	18.4 ± 0.8 b	80.2 ± 5.2 a	3.9 ± 0.3 b	0.29 ± 0.1 a
<i>S. eridania</i> on Cry1F	1.1 ± 0.5 c	0.0 ± 0.0 c	³ -	-
<i>S. frugiperda</i> on Cry1F	1.6 ± 0.9 c	0.0 ± 0.0 c	-	-
<i>S. eridania</i> on Cry1F + Cry1A.105 + Cry2Ab2	0.9 ± 0.5 c	0.0 ± 0.0 c	-	-
<i>S. frugiperda</i> on Cry1F + Cry1A.105 + Cry2Ab2	1.3 ± 0.8 c	0.0 ± 0.0 c	-	-
CV (%)	5.31	11.49	15.4	6.6
DF residual	24	24	8	8
F	42.8	91.32	10.3	7.3
P	≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05

¹Means ± SEM followed by the same letter in the same stage of development of the insect column do not differ by Tukey test ($P \leq 0.05$).

²Means ± SEM followed by the same letter in the column do not differ by *t*-test ($P \leq 0.05$).

³Parameter non existent.

study is similar to that of *S. eridania* observed on suitable hosts, such as cotton bolls and morning glory (SANTOS et al., 2005). So, is possible that the higher consumption of corn cobs might be associated with toxins or nutritional imbalance present in the food (LARA, 1991), suggesting a poor quality to larval development of *S. eridania*. In this sense, the lower pupal biomass of *S. frugiperda* could be attributed to the biological differences between the species and adaptation to consume ears of field corn.

Efficiency of Cry1F protein in controlling *S. frugiperda* has been reported in other studies (BUNTIN, 2008; SIEBERT et al., 2008), but it was observed when to *S. frugiperda* were offered corn leaves only. There fore, this is the first study in which insecticide proteins (single Cry1F and pyramided Cry1F + Cry1A.105 + Cry2Ab2) expressed in fresh corn cobs helped to kill *S. frugiperda* like thenon-target pest *S. eridania*. There was genetic variability in different populations that can show resistance to maize *Bt*. For example, FARIAS et al. (2014) recently reported *S. frugiperda* populations that were resistant against Cry1F protein expressed in corn leaves. It is crucial to adopt integrated resistance management IRM, monitoring pest infestation in crop fields, and adopting a refuge area to less risks of increase resistance.

Overall, although both *Bt* events showed similar effects in *Spodoptera* spp. larvae, it is usually recommended to prefer pyramided events because of the low risk of resistant development. This is reinforced by a previous study that showed reduced

damage of corn cobs by *S. frugiperda* in *Bt* pyramided (expressing Cry1A.105 e Cry2Ab2) cornfields when compared with single *Bt* corn Cry1A(b) (WAQUIL et al., 2013). Thus, *Bt* pyramided corn allowed to reduce the refuge area in which the number of pests that survive is lower than that in a field which a single *Bt* corn has been cultivated (SIEBERT, 2012). Finally, this study indicated that Cry1F and Cry1F + Cry1A.105 + Cry2Ab2 expressed in corn cobs helped to control *S. eridania* and *S. frugiperda* larvae. However, non-*Bt* corn fields should be constantly monitored, since these larvae have the potential to damage corn plants.

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