Revista Brasileira de Entomologia 60 (2016) 255–259

SOCIEDADE BRASILEIRA DE ENTOMOLOGIA
FUNDADA EM 1937

REVISTA BRASILEIRA DE
Entomologia
A Journal on Insect Diversity and Evolution

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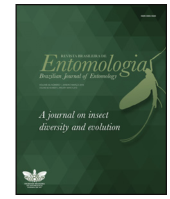

Biological Control and Crop Protection

Biological characteristics of black armyworm *Spodoptera cosmioides* on genetically modified soybean and corn crops that express insecticide *Cry* proteins

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ARTICLE INFO

Article history:
Received 13 August 2015
Accepted 29 April 2016
Available online 24 May 2016
Associate Editor: Daniel Ricardo Sosa Gómez

Keywords:
Black armyworm
Genetically modified organisms
Non-target pests
Plant resistance

ABSTRACT

This study aimed to evaluate the development and reproduction of the black armyworm, *Spodoptera cosmioides* when larvae fed on leaves of *Bt*-corn hybrids, expressing a single Cry1F and also Cry1F, Cry1A.105 and Cry2Ab2 in pyramided corn and their non-*Bt*-isoline (hybrid 2B688), as well as on leaves of two soybean isolines expressing the Cry1Ac protein and its non-*Bt* isoline (A5547-227). We also assessed the effect of these *Bt* and non-*Bt* plants on the leaf consumption rate of *S. cosmioides* larvae. This pest was unable to develop when fed on any of the corn isolines (*Bt* and non-*Bt*). When both 1st and 3rd instar larvae were fed on corn leaf, mortality was 100% in both *Bt* and non-*Bt* corn. In contrast, when corn leaves were offered to 5th instar larvae, there were survivors. Defoliation and leaf consumption was higher with non-*Bt* corn than with both of the *Bt* corn isolines. There was no negative effect of *Bt* soybean leaves on the development and reproduction of *S. cosmioides* with respect to all evaluated parameters. Our study indicates that both *Bt* and non-*Bt* corn adversely affect the development of *S. cosmioides* while *Bt* soybean did not affect its biology, suggesting that this lepidopteran has major potential to become an important pest in *Bt* soybean crops.

Introduction

The black armyworm, *Spodoptera cosmioides* (Walker, 1858) (Lepidoptera: Noctuidae), previously a synonym for the North and Central American species *Spodoptera latifascia* Walker, 1856 (Lepidoptera: Noctuidae), is a strictly polyphagous South American species (Silvain and Lalanne-Cassou, 1997). It occurs both on cultivated plants and weeds and might cause severe yield reduction to various crops of economic importance (Bavaresco et al., 2004; Habib et al., 1983). This pest defoliates soybean plants throughout the crop cycle, and is twice as effective as other lepidopteran pests that attack this crop (Bueno et al., 2011). It also feeds on soybean pods (Gazzoni and Yorinori, 1995).

Cropping of plants (soybean, corn among others) that express *Cry* proteins from *Bacillus thuringiensis* Berliner is increasing worldwide (James, 2013) not only due to their high pest control efficacy but also because of their ease of cultivation. These genetically modified plants express proteins from the *Cry* genes targeting Lepidoptera and Coleoptera pests (Head et al., 2014; Praça et al., 2004). *Bt*-corn, frequently in combination with genetic modification for herbicide tolerance, has been widely cultivated worldwide for many years (James, 2013) to control the fall armyworm *Spodoptera frugiperda* (J. E. Smith, 1797), the stem borer *Diatraea saccharalis* (Fabricius, 1794) (Braga et al., 2003), the corn earworm *Helicoverpa zea* (Boddie, 1850) (Chilcutt et al., 2007), and other caterpillars. In contrast, the first commercialized *Bt* soybean was developed by Monsanto by combining the transformation events MON 87701 (expressing Cry1Ac protein) and MON 89788 (glyphosate tolerance) in the same plant. It was commercially released in Brazil in 2010 and in Argentina in 2012 for control of major soybean caterpillars (Bortolotto et al., 2014).

Some studies have reported that the adoption of *Bt* crops leads to a reduction in insecticide use (Hutchison et al., 2010; Kouser and Qaim, 2011; Lu et al., 2012; Sisterson et al., 2007). It may favor the increase of biological control agents that are eliminated by the abusive use of non-selective pesticides (Sisterson et al., 2007).

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http://dx.doi.org/10.1016/j.rbe.2016.04.005

Table 1
Biological parameters of *Spodoptera cosmioides* fed on *Bt* and non-*Bt* crops.

Trial[a]	Plant	Cultivars	Proteins	Developmental stage	Evaluated parameters
1	corn	2B688HX 2B688PW 2B688	Cry1F Cry1F + Cry1A.105 and Cry2Ab2 –	1th instar	Days of survival and mortality (%)
2	corn	2B688HX 2B688PW 2B688	Cry1F Cry1F + Cry1A.105 and Cry2Ab2 –	3th instar	Days of survival and mortality (%)
3	corn	2B688HX 2B688PW 2B688	Cry1F Cry1F + Cry1A.105 and Cry2Ab2 –	5th instar	Duration of each instar (days), mortality (%) and total leaf consumption of 5th + 6th instar
4	soybean	MON 87701 × MON 89788 A5547-227	Cry1Ac –	1th instar	Duration of larval and pre-pupal stages (days), pupal weight (g), sex ratio, survival (%) larvae-adults, leaf consumption (cm^2), fecundity and longevity of *S. cosmioides* descendants as well as their egg viability.

[a] Different bioassays carriet out in this study.

However, the elimination of interspecific competitions can also favor certain secondary pest outbreaks which are not controlled by *Bt* plants (Zhao et al., 2011). Therefore, it is important to understand the direct and indirect impact of *Bt* plants on non-target pest species, such as *S. cosmioides*. Thus, the present study aimed to evaluate the influence of *Bt* soybean and *Bt* corn on consumption, development and reproduction of the non-target pest *S. cosmioides*.

Material and methods

This study is composed of four independent bioassays (Table 1). All trials were carried out in the laboratory under controlled environmental conditions (25 ± 2 °C, RH of 70 ± 10%, photoperiod of 14:10 h [L:D]). The first trial (bioassay 1) compared the performance of the caterpillar *S. cosmioides* of first instar on *Bt*-corn (2B688 Herculex® and 2B688 PowerCore®) and its non-*Bt* isoline (2B688). The second trial (bioassay 2) was similar to bioassay 1, but in this case, evaluations were done on third instar larvae [first and second instar were kept at artificial diet as described by Pomari et al. (2012)]. The third trial (bioassay 3) compared the performance and the leaf consumption of 5th-instar *S. cosmioides* on both *Bt* (2B688 Herculex® and 2B688 PowerCore®) and non-*Bt* corns (2B688). Fourth trial (bioassay 4) compared the performance of the caterpillar *S. cosmioides* of first instar on *Bt*-soybean (MON 877701 × MON 89788) and non-*Bt* near isoline (A5547-227).

Insects and plant origin

S. cosmioides was reared in the laboratory according to the method described by Pomari et al. (2012) for approximately 34 generations under controlled environmental conditions (25 ± 2 °C, RH of 70 ± 10%, photoperiod of 14:10 h [L:D]).

The corn products tested were Herculex® (expressing Cry1F protein, 2B688HX) and PowerCore® (expressing Cry1F, Cry1A.105 and Cry2Ab2 proteins, 2B688PW), as well as their non-*Bt* isoline (hybrid 2B688). These seeds were developed and provided by Dow AgroSciences Ltda.

Soybean seeds were from two soybean isolines *Bt*-soybean MON 87701 × MON 89788 (cv. Intacta RR2 PRO), expressing the Cry1Ac protein and its non-*Bt* isoline (A5547-227). These seeds were developed and provided by Monsanto Ltda.

Both corn and soybean plants were grown in plastic pots (volume 8 L) in a greenhouse. Powdery mildew was controlled with a sulfur-based fungicide (Kumulus® 0.5 $g\,l^{-1}$) that was applied weekly.

Comparative S. cosmioides *leaf consumption, development and reproduction on* Bt *and non-*Bt *corn (bioassays 1, 2, and 3)*

The independent experiments were carried out in a completely randomized design with three treatments (*Bt* Herculex® and PowerCore® and non-*Bt* corn isoline) and ten replicates, with each replicate containing eight individualized larvae of *S. cosmioides* (totaling 80 larvae per treatment).

In bioassay 1, newly hatched larvae (up to 24 h old) were individualized in transparent plastic pots (150 mL). The pot lids had small holes to ensure airflow to both caterpillars and leaves. Water was provided by small cotton balls placed on each leaf to avoid excessive dehydration.

Corn leaves were harvested daily from plants cultivated in greenhouse and cleaned for approximately 15 min in a 5% sodium hypochlorite solution, and then dried for 2 h before feeding them to *S. cosmioides* larvae. In all cases, the second expanded leaf (from the top of the plant) was used. Several corn seeds were sown on a daily basis to ensure the availability of leaves required for this experiment. Instar and survival of *S. cosmioides* larvae were recorded daily. Since we observed 100% mortality of all 1st instar *S. cosmioides* larvae, regardless of whether they were feeding on *Bt* or non-*Bt* corn leaves, the experiment was restarted (bioassay 2) using 3rd instar larvae.

Prior to the second experiment (bioassay 2) larvae were reared on an artificial diet described by Hoffmann-Campo et al. (1994) until reaching the 3rd instar. Using this instar, the experiment was carried out following the methodology previously described. Again, 100% mortality was recorded for all *S. cosmioides* larvae regardless of whether they were feeding on *Bt* or non-*Bt* corn leaves. Therefore, a new experiment was carried out (bioassay 3) using 5th instar larvae.

In the bioassay 3, 5th instar larvae lived longer, thus allowing the measurement of leaf consumption. *S. cosmioides* instars, lifespan and larval survivorship were recorded daily. Leaf-feeding on *Bt* and non-*Bt* corn was measured using a leaf area meter (Model LI-3100, Li-Cor, Lincoln, NE) before and after larval feeding. The daily foliage consumption by each specimen was then calculated by subtracting the final (defoliated) from the initial (offered) leaf area. During the entire experiment (5th and 6th instar), a control leaf was used to estimate leaf dehydration and the related reduction in leaf size. Leaf area (in cm^2) of the control leaves was measured daily, and reduction in leaf size used to adjust the results for daily larvae consumption. Total consumption by individual larvae was recorded for each specimen, and the average consumption obtained from each replicate was used for analysis (Bueno et al., 2011).