



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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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.

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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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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 <i>S. cosmioides</i> descendants as well as their egg viability.

^a Different bioassays carried 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* isolate (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 isolate (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* isolate (hybrid 2B688). These seeds were developed and provided by Dow Agro-Sciences 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* isolate (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 isolate) 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 cm²) 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).

Comparative *S. cosmioides* leaf consumption, development and reproduction on *Bt* and non-*Bt* soybean (bioassay 4)

A similar methodology as previously described in the experiments with corn was used in a single experiment to compare *S. cosmioides* leaf consumption, development and reproduction on *Bt* and non-*Bt* soybean. The experiment (bioassay 4) was carried out in a completely randomized design with two treatments (*Bt* and non-*Bt* soybeans) and ten replicates, with each replicate containing 8 individualized larvae of *S. cosmioides* (totaling 80 larvae per treatment). 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 placing small cotton balls on the stem of each leaf to avoid excessive dehydration.

Soybean leaves were excised daily from plants at the V7 developmental stage (Fehr and Caviness, 1977) and cleaned for approximately 15 min in a 5% sodium hypochlorite solution, and 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 soybean seeds were sown on a daily basis to ensure the availability of leaves required for this experiment.

S. cosmioides instars, lifespan, larval survivorship, adult longevity, fecundity and egg viability were recorded daily. Within 24 h after caterpillars had pupated, each individual was sexed and weighed with 0.001 g precision. To assess parameters of the adult stage, <24 h old moths were paired. After mating, female moths were placed in enclosures made of plastic pipes (10 cm in diameter × 21.5 cm tall), that on the inner surface were lined with white paper to allow oviposition. A cotton wad soaked with a 10% honey solution was placed inside each enclosure to feed the moths.

Leaf-feeding on *Bt* and non-*Bt* soybeans was measured when caterpillars reached the 5th instar until pupation. For this, soybean foliage area (cm²) was determined with the previous methodology. During the entire period of evaluation (5th instar to pupae) a control leaf of each soybean isolate was measured to estimate leaf dehydration and the resulting reduction in leaf size. The leaf area (in cm²) of the control leaf was measured daily, and the reduction in leaf size used to adjust the results for daily larvae consumption. Total consumption by individual larvae was recorded for each specimen, and average consumption obtained from each replicate was used for analysis (Bueno et al., 2011).

Statistical analysis

The bioassay results were subjected to exploratory analyses to assess the assumptions of normality of residuals (Shapiro and Wilk, 1965), homogeneity of variance of treatments and additivity of the model (Burr and Foster, 1972). Data not following the normality assumptions or homogeneity of variance were transformed. Sex ratio and egg viability data from *S. cosmioides* biology feeding on *Bt* and non-*Bt* soybean leaves required transformation to $\sqrt{X+1}$ for ANOVA. Means were then compared by Tukey test for corn trials or Student's *t*-test for soybean trials ($p \leq 0.05$) (SAS Institute, 2001).

Results

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

Survivorship of 1st instar larvae did not differ between non-*Bt*, Herculex[®] and PowerCore[®] (1.7, 1.5 and 1.6 days, respectively). In contrast, when larvae were reared on an artificial diet until

Table 2

Days of survival of 1st and 3rd instar of *Spodoptera cosmioides* (Lepidoptera: Noctuidae) fed on *Bt* and non-*Bt* corn [followed by mortality %].

Corn	Survival (days) ^a [Mortality %]	
	1st instar (bioassay 1)	3rd instar (bioassay 2)
2B688 (non- <i>Bt</i>)	1.7 ± 0.1 ^b [100] ^c	5.7 ± 0.2 a [100]
2B688 Herculex [®]	1.5 ± 0.1 [100]	4.0 ± 0.1 c [100]
2B688 PowerCore [®]	1.6 ± 0.1 [100]	4.7 ± 0.1 b [100]
CV (%)	11.93	10.29
DF _{residue}	27	27
F	2.1	29.59
p	0.142	0.0001

^a Means ± SEM followed by the same letter within columns did not significantly differ from each other (Tukey test, $p > 0.05$).

^b ANOVA not significant.

^c Values between brackets are the percentage of insect mortality.

completing the 2nd instar and then fed with corn leaves beginning from the 3rd instar onwards, they lived longer on non-*Bt* corn leaves (5.7 days) compared with *Bt* treatments (4.0 and 4.7 days for Herculex[®] and PowerCore[®], respectively). However, we recorded 100% mortality of *S. cosmioides* larvae for both 1st and 3rd instar, regardless of their food (*Bt* or non-*Bt* corn leaves, Table 2).

When caterpillars were reared on an artificial diet until completing the 4th instar and then fed with corn leaves from the 5th instar onwards, higher mortality was observed for *Bt* treatments (20.0 and 22.9% for Herculex[®] and PowerCore[®], respectively) than for non-*Bt* corn (14.3%). Although at the 5th instar there was no difference in lifespan, mortality reached 100% at the 6th instar, regardless of the food offered (*Bt* or non-*Bt* corn leaves, Table 3). However, despite 100% mortality at the 6th instar, we were able to measure leaf consumption at the 5th and 6th instar. Leaf consumption rates were higher for non-*Bt* leaves (52.32 cm²) than for *Bt* treatments (27.35 and 21.87 cm² for Herculex[®] and PowerCore[®], respectively; Table 3).

Comparative *S. cosmioides* leaf consumption, development and reproduction on *Bt* and non-*Bt* soybean (bioassay 4)

Lifespan of *S. cosmioides* larvae (means ± SEM of 33.0 ± 0.3 and 34.0 ± 0.4 days when feeding on non-*Bt* and *Bt*-soybean, respectively) and pre-pupae (means ± SEM of 1.9 ± 0.2 days when feeding on both non-*Bt* and *Bt*-soybean), pupae weight (means ± SEM of 0.26 ± 0.1 and 0.23 ± 0.1 grams when feeding on non-*Bt* and *Bt*-soybean, respectively) and sex ratio (means ± SEM of 0.33 ± 0.1 and 0.60 ± 0.2 when feeding on non-*Bt* and *Bt*-soybean, respectively) did not differ, regardless of the food offered (*Bt* or non-*Bt* soybeans). Similarly, results for larval survival (means ± SEM of 82.2 ± 2.5% and 82.8 ± 1.8% when feeding on non-*Bt* and *Bt*-soybean, respectively), which was higher than 80%, as well as for foliar consumption (means ± SEM of 133.9 ± 18.8 cm² and 150.6 ± 12.8 cm² when feeding on non-*Bt* and *Bt*-soybean, respectively) did not differ between the two treatments.

Moreover, adult fecundity (means ± SEM of 903.69 ± 96.35 eggs and 892.36 ± 94.14 eggs when feeding on non-*Bt* and *Bt*-soybean, respectively) and longevity (means ± SEM of 13.3 ± 0.6 days and 12.8 ± 0.6 days for males and 14.3 ± 1.0 days and 13.1 ± 0.7 days for females when feeding on non-*Bt* and *Bt*-soybean, respectively), as well as egg viability (means ± SEM of 56.71 ± 7.22% and 52.27 ± 6.13% when feeding on non-*Bt* and *Bt*-soybean, respectively) did not differ between both *Bt* and non-*Bt* soybeans. Thus, *S. cosmioides* leaf consumption and performance on *Bt* and non-*Bt* soybeans were similar, confirming that *Bt* soybean MON 87701 × MON 89788 consumption did not control *S. cosmioides* nor impact its biology.

Table 3
Duration (days) and leaf consumption of 5th instar *Spodoptera cosmioides* (Lepidoptera: Noctuidae) fed on *Bt* and non-*Bt* corn.

Corn	Duration (days) ^a [Mortality %]		Leaf consumption ^a 5th and 6th instars (cm ²)
	5th instar	6th instar	
2B688(non- <i>Bt</i>)	4.9 ± 0.1 ^c [14.3]	– ^b [100]	52.32 ± 2.35 a
2B688 Herculex [®]	4.7 ± 0.1 [20.0]	– [100]	27.35 ± 1.81 b
2B688 PowerCore [®]	4.6 ± 0.1 [22.9]	– [100]	21.87 ± 1.48 b
CV (%)	7.4	–	6.2
DF _{residue}	18	–	12
F	1.2	–	68.21
p	0.3229	–	0.0001

^a Means ± SEM followed by the same letter within columns did not significantly differ from each other (Tukey test, $p > 0.05$).

^b Non-existent parameter.

^c ANOVA not significant.

Discussion

The previously reported efficient control of *Spodoptera frugiperda*, the main corn pest in South America (Fernandes et al., 2003; Waquil et al., 2002), by *Bt* corn raised the question if this drastic decrease of *S. frugiperda* could favor *S. cosmioides* outbreaks by the elimination of a possible interspecific competition between these species. The elimination of a secondary species by a dominant species on various plants is usually related to intraguild competition (Cassino et al., 1993), which may also be the case among *Spodoptera* species. However, although *S. cosmioides* consumed a large amount of leaf tissue of both *Bt* and non-*Bt* corn during the 5th and 6th larval instar, this species will probably not inflict economic damage on corn crops because larvae fail to reach the adult stage. Therefore, even though classified as a polyphagous species that feeds on both cultivated plants as well as on weeds (Bavaresco et al., 2004) and reported in attacks to corn plants (Figueiredo et al., 2011), *S. cosmioides* is unable to complete its development when feeding exclusively on corn leaves. This was shown independently for both *Bt* and non-*Bt* corn leaves.

Growers point out that *S. cosmioides* may migrate from weeds to corn plants at a more advanced instar, and thus succeed in attacking *Bt*-corn. Under field conditions, *S. cosmioides* larvae feed on leaves of different host plants, such as weeds commonly found in corn fields. Herbicide application that kills these hosts forces the larvae to migrate to cultivated plants. Even though corn plants are not regarded as a suitable host, more advanced stages of caterpillars are capable of defoliating corn plants as shown in our study. This hypothesis needs to be further investigated under field conditions. We suggest that weed management is likely to play an important role in pest management. Nevertheless, our results also show that consumption of *Bt*-corn leaves by 5th and 6th instar larvae of *S. cosmioides* was approximately 50% less than their consumption of non-*Bt* maize leaves, suggesting that the potential of this insect-pest to cause significant damage to *Bt* corn is limited.

S. cosmioides is a soybean pest and, in contrast to results recorded for corn leaves, development and reproduction on this crop was not affected by the Cry1Ac protein. Although the Cry1Ac protein, expressed in *Bt* soybean MON 87701 × MON 89788, is specific to lepidopterans (CTNBio, 2010), *S. cosmioides* was not affected in our study. Our results on leaf consumption as well as the lack of an effect of *Bt* soybean on the biological characteristics during larval and adult stages support the idea that the level of activity of the Cry1Ac protein against *S. cosmioides* is low. The lack of an effect of Cry1Ac on *Spodoptera* has been previously reported (Greenberg et al., 2010; Santos et al., 2009) and may be related to a natural high tolerance of this insect-pest to the Cry1Ac protein (Maagd et al., 2000). Weak binding of Cry1Ac to the midgut (Aranda et al., 1996), or the inactivation of the insecticidal proteins by proteases produced by the insects (Miranda et al., 2001; Rahman et al., 2012) have been reported for larvae of other species, such as *S. frugiperda*

and *S. exigua*. Plant developmental stage may also play an important role in the efficacy of the Cry protein. For example, larval survival of *S. exigua* was not affected by feeding on *Bt* soybean plants throughout the growing season, but larval weight was reduced when larvae fed on leaves of two *Bt* soybean varieties before the anthesis stage, when Cry1Ac concentrations were at a maximum (Yu et al., 2013).

Susceptibility to *B. thuringiensis* varies greatly among different species (Schnepf et al., 1998) which confirms the importance of analyzing *Bt* impact on target and non-target insects. In this context, in addition to direct effects of *Bt* plants on pest performance and behavior, some indirect effects may occur. *Bt* crops may facilitate the reduction of insecticide use to control pest outbreaks (Sisterson et al., 2007), as reported after the adoption of *Bt* cotton in Arizona (Carpenter and Gianessi, 2001; Cattaneo et al., 2006). It is likely that insecticide use on soybeans can be reduced where MON 87701 × MON 89788 is grown.

Based on the results of our experiments, we conclude that *Bt* soybean MON 87701 × MON 89788 had no effect on *S. cosmioides* development and reproduction, and that soybean was a good host for the development of the pest allowing for more than 80% larvae-to-adult survival. Therefore, this lepidopteran has major potential to become an important pest in *Bt* soybean crops. In contrast, larvae of *S. cosmioides* were not able to complete their larval stage when fed on either *Bt* or non-*Bt* corn leaves, which suggests its non-suitability as a host for this pest.

Conflicts of interest

The authors declare no conflicts of interest.

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