

Impact of Cry1Ac soybean (*Glicine max*) on biological and reproductive cycles and herbivory capacity of *Spodoptera cosmioides* and *Spodoptera eridania* (Lepidoptera: Noctuidae)

Impacto de la soja (*Glicine max*) Cry1Ac sobre el ciclo biológico, reproductivo y la capacidad herbívora de *Spodoptera cosmioides* y *Spodoptera eridania* (Lepidoptera: Noctuidae)

Alejandra Liliana Lutz ^{1*}, Laura Noemí Fernandez ^{1,2}, Verónica Eugenia Ruiz ²,
María Cecilia Curis ¹, Melina Soledad Buttarelli ³, Pablo Daniel Sánchez ¹,
María Alejandra Favaro ^{1,2}, Roberto Ricardo Scotta ¹

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ABSTRACT

Increasing populations of *Spodoptera cosmioides* (Walker) and *Spodoptera eridania* (Stoll) have recently been detected in soybean crops in central Argentina. Besides being polyphagous, these species tolerate the Cry1Ac insecticidal toxin, expressed by genetically modified *Bt* soybean (MON89788 x MON87701). Consequently, when facing big populations, farmers often apply insecticides. This study aimed to determine the effects of *Bt* soybean on the consumption, biological cycle, and reproduction of both *Spodoptera* species. Larval feeding on *Bt* soybean led to a shorter pupal period (23% less than control) and a decreased leaf-area consumption for *S. cosmioides* (14% less than the non-*Bt* soybean). In *S. eridania*, the larval stage, adult longevity, larva-to-adult, and oviposition periods were reduced (11, 23, 13, and 30% shorter than control, respectively). Despite these reductions, both Lepidoptera species completed their reproductive cycles. These valuable findings help us understand the biology of these potential pests in *Bt* soybean crops in Argentina.

Keywords

Glicine max (L.) • plant resistance • non-target pests • black armyworm • southern armyworm

1 Universidad Nacional del Litoral. Facultad de Ciencias Agrarias. Departamento de Producción Vegetal. Kreder 2805 (3080). Esperanza. Santa Fe. Argentina.

* alutz@fca.unl.edu.ar

2 Universidad Nacional del Litoral. Facultad de Ciencias Agrarias. ICiAgro Litoral. CONICET, Esperanza. Argentina.

3 Estación Experimental Agropecuaria Rafaela (INTA E.E.A. Rafaela). Área de producción Vegetal. Ruta 34 km 227 (2300). Rafaela. Santa Fe. Argentina.



RESUMEN

En los últimos años, las poblaciones de *Spodoptera cosmioides* (Walker) y *Spodoptera eridania* (Stoll) se han incrementado en los cultivos de soja de la zona central de Argentina. Además de ser polífagas, estas especies son tolerantes a la toxina insecticida Cry1Ac expresada por la soja *Bt* genéticamente modificada (MON89788 x MON87701), por lo que los agricultores deben recurrir al control químico con insecticidas cuando se presentan altas densidades poblacionales. Este estudio tuvo como objetivo determinar el efecto de la soja *Bt* sobre el consumo, ciclo biológico y reproducción de ambas especies de *Spodoptera*. La alimentación larval con soja *Bt* determinó una menor duración del período pupal (23% menos que el tratamiento control) y una disminución en el consumo de área foliar en *S. cosmioides* (14% menos que la soja no *Bt*). *Spodoptera eridania* registró una menor duración del estado larval, longevidad de adultos, período larva-adulto y del período de oviposición (11, 23, 13 y 30% menos que el tratamiento control, respectivamente). Sin embargo, ambas especies de *Lepidoptera* completaron su ciclo reproductivo con éxito. Los resultados obtenidos en este trabajo son de gran utilidad para comprender la biología de estas especies, que tienen el potencial de convertirse en plagas importantes en los cultivos de soja *Bt* en Argentina.

Palabras clave

Glicine max (L.) • plantas resistentes • plagas no blanco • oruga cogollera negra • oruga cogollera del sur

INTRODUCTION

Genetically modified (GM) crops exhibiting insect-resistance are valuable tools in integrated pest management (IPM) systems (25). These crops express genes derived from the entomopathogenic bacterium *Bacillus thuringiensis* Berliner (*Bt*), producing (Cry) proteins with highly selective insecticidal activity. *Bt* soybean expressing these insecticidal toxins is effective in controlling several major lepidopteran pests in agricultural environments, including *Anticarsia gemmatilis* (Hübner), *Chrysodeixis includens* (Walker), *Helicoverpa gelotopoeon* (Dyar) and *Rachiplusia nu* (Guenée) (44).

High efficacy of *Bt* soybean crops against pest populations and the consequent reduced insecticide use has significantly altered the agroecosystem. Consequently, the reduced interspecific competition after controlling *Bt* target species has facilitated the emergence of new phytophagous pest species; many of which could become economically significant (19, 30, 47). Recent reports mention increasing populations of *Spodoptera cosmioides* Walker and *Spodoptera eridania* Stoll (*Lepidoptera*: *Noctuidae*) in Argentinean soybean crops, including *Bt* cultivars (23, 29, 30, 32). Factors contributing to these phenomena include tolerance to the Cry1Ac protein, insecticide resistance and the ability to complete life cycles on the weed *Amaranthus* sp. (2, 5, 9, 24, 26, 30). *S. eridania* thrives in temperate regions like the Argentinean Pampas, with a developmental threshold of 11.9°C and an inability to complete its life cycle above 34°C. In contrast, *S. cosmioides* is adapted to warmer temperatures (from 13.2), prevailing in soybean and cotton crops in northern Argentina (31). Although soybean and cotton are the preferred hosts (11), caterpillars of both species are polyphagous, and develop on weeds and grain, fruit, and ornamental crops (14). These species also have greater herbivorous potential than other soybean defoliators, consuming vegetative structures, flowers, and pods (6, 21, 27, 38).

Understanding biological and reproductive pest cycles is essential for elucidating population dynamics and predicting potential crop populations. Most studies assessing the effects of the Cry1Ac protein on the development and foliar consumption of lepidopteran pests (4, 5, 8, 12) have been conducted in Brazil, with almost no equivalent in Argentina. This study hypothesized that the Cry1Ac protein affects biological performance, reproduction, and feeding behavior of both *Spodoptera* species. We investigated the impact of *Bt* soybean on foliar consumption and life cycle to assess pest potential in the central soybean region of Argentina.

MATERIALS AND METHODS

This study was conducted in the breeding chamber of the Plant Production Department at the Facultad de Ciencias Agrarias (Universidad Nacional del Litoral), in Esperanza City, Santa Fe province, Argentina.

Insect rearing

Spodoptera cosmioides and *S. eridania* larvae were collected in February 2019 from commercial soybean fields in Santa Fe province, near Franck (31°35'00"S 60°56'00"W, 31 m.a.s.l.) and Santa María Norte (31°31'00"S 61°08'00"W, 44 m.a.s.l.). The caterpillars were transported to the breeding chamber in containers with soybean leaves and identified using taxonomic keys (43, 46). They were reared under controlled temperature ($24 \pm 2^\circ\text{C}$), relative humidity (60%), and photoperiod (14:10 h, light: dark) in transparent PVC boxes (26 cm long, 17 cm wide, and 7 cm high), covered with muslin caps for air circulation. An artificial diet consisting of corn flour, wheatgerm, yeast, water, agar, nipagin, benzoic acid, and ascorbic acid was provided until pupation (33). The emerged adults were placed in oviposition cages (50 cm length, 40 cm width, and 40 cm height), with paper sheets for oviposition. They were fed daily with an artificial adult diet (10) provided through soaked cotton. Eggs were collected daily and placed in 9 cm diameter Petri dishes with artificial feed for neonate larvae. Three days after hatching, larvae were transferred to PVC boxes for large-scale rearing with an artificial diet. This process continued until the F_2 generation, ensuring enough population for the study.

Plant material

Leaves for larvae feed were obtained from soybean cultivars RA 5715 IPRO (*Bt*) and RA 549 (non-*Bt*). Both cultivars are glyphosate-tolerant, but only the former expresses the Cry1Ac toxin. To ensure a continuous supply of leaves, both cultivars were periodically planted in 3x2 m plots under field conditions. Weeds were manually removed and soybean plants were kept disease-free by the eventual application of fungicides.

Effect of *Bt* soybean on the biological and reproductive cycle of *S. cosmioides* and *S. eridania*

A second instar (L2) larva of either *S. cosmioides* or *S. eridania* was placed on two soybean leaflets (from *Bt* or non-*Bt* soybean plants, depending on the treatment) inside 9 cm diameter Petri dishes lined with absorbent paper. Petioles were wrapped in cotton saturated with distilled water, maintaining humidity. *Bt* and non-*Bt* soybean leaflets were harvested at V6-V8 vegetative stage before anthesis, according to phenology by Fehr *et al.* (1977). The V6-V8 vegetative stage corresponds to maximum Cry1Ac expression in the *Bt* cultivar (45). Food and absorbent papers were renewed daily until pupation. We defined sex by observing the terminal portion of pupae (7) using a stereomicroscope set (Lancet Instruments, China) at 30× magnification. Thirty replicates were performed for each treatment (*Bt* and non-*Bt* soybean) and species (*S. cosmioides* and *S. eridania*). Once adults emerged, one couple was placed per oviposition container (17 cm height, 11 cm upper diameter, and 7 cm lower diameter), covered with a muslin cap facilitating air circulation and preventing adult escape. The same diet used for rearing was supplied to adults with soaked cotton (10). Fecundity was determined by daily collecting egg masses laid by females after mating. Egg masses were photographed using an Olympus SZ40 stereomicroscope (Olympus Corporation, Tokyo, Japan) at 40X for egg counting, considering any overlapping or superimposed eggs. Each egg mass was placed in a separate Petri dish (9 cm diameter) lined with absorbent paper and food for emerging neonates. Fertility (viable eggs) was estimated by the number of viable larvae hatched from each egg mass. The assays were conducted with 11 and 10 couples of *S. cosmioides* and 14 and 10 couples of *S. eridania* for the *Bt* and non-*Bt* soybean treatments, respectively.

The following variables were recorded: duration (in days) of larval, pupal and adult stages, the larva-to-adult period, pupal weight (g) using an OHAUS-PIONNER precision scale (± 0.0001 g), fecundity (number of eggs/female), fertility (number of hatched eggs), pre-oviposition (days from adult emergence to first egg laying), oviposition (days from the first to the last egg laying), and post-oviposition (days from last egg laying to death).

Effect of *Bt* soybean on leaf consumption by larvae of *S. cosmioides* and *S. eridania*

Leaf area consumption (cm²) was determined using the same larvae and soybean cultivars (species and treatments) as when assessing the impact of *Bt* soybean on the biological and reproductive cycle. Fresh leaflets were provided daily as food, and the remaining unconsumed portions were scanned using an HP Deskjet F4280 multifunction printer. The consumed leaf area was quantified by image analysis with ImageJ® software (1). Adjusted leaf area loss due to dehydration was based on data from soybean leaflets not exposed to larvae.

Statistical analysis

Bioassays for each lepidopteran species were conducted independently under a completely randomized experimental design. Since the duration of larva, pupal, and adult stages, as well as the larva-to-adult period did not meet normality, non-parametric Kruskal-Wallis test ($\alpha \leq 0.05$) was performed. Pupal weight was analyzed by ANOVA and Tukey test ($\alpha \leq 0.05$). Foliar consumption

means were compared using an independent samples T-test ($\alpha \leq 0.05$). All statistical analyses were conducted using InfoStat software (13).

RESULTS**Effect of *Bt* soybean on the biological and reproductive cycle of *S. cosmioides* and *S. eridania***

Feeding *S. cosmioides* larvae with *Bt* soybean leaves did not significantly affect larval duration compared to control, with 20.46 and 19.48 days, respectively ($H = 0.64$; $p = 0.4160$) (table 1). However, in *S. eridania*, significant differences were evidenced for larval length of 28.13 days when fed with *Bt* soybean leaves and 31.69 days when supplied with non-*Bt* soybean leaves ($H = 7.43$; $p = 0.0062$) (table 1).

Significant differences in the *S. cosmioides* pupal stage duration showed 10.65 and 13.96 days ($H = 14.72$; $p = 0.0001$), with *Bt* and non-*Bt* soybean, respectively. In *S. eridania*, differences were not significant ($H = 3.77$; $p = 0.0434$) (table 1). Regarding the adult stage, no significant differences were found in *S. cosmioides* ($H = 0.04$; $p = 0.8485$). However, *S. eridania* adults lived significantly longer on non-*Bt* soybean leaves (12.87 days), compared to *Bt* soybean (9.90 days) ($H = 11.70$; $p = 0.0005$) (table 1).

Table 1. Days of larval, pupal, and adult stages, larva-to-adult period, and pupae weight (g) (Mean $\pm$ SD) of *Spodoptera cosmioides* and *S. eridania*, fed *Bt* and non-*Bt* soybean leaves under controlled conditions.

Tabla 1. Duración (días) de los estadios larval, pupal y adulto, el período de larva a adulto y el peso de las pupas (g) (Media $\pm$ DE) de *Spodoptera cosmioides* y *S. eridania*, alimentadas con hojas de soja *Bt* y no-*Bt* en condiciones controladas.

<i>Spodoptera cosmioides</i>						
Treatment	n	Larva (days)*	Pupae (days)*	Adult (days)*	Larva-to-adult period (days)*	Pupae weight (g)*
<i>Bt</i>	26	20.46 $\pm$ 3.37 a	10.65 $\pm$ 3.57 a	13.19 $\pm$ 4.92 a	44.31 $\pm$ 9.12 a	0.33 $\pm$ 0.08 a
non- <i>Bt</i>	23	19.48 $\pm$ 2.0 a	13.96 $\pm$ 3.93 b	13.26 $\pm$ 5.14 a	46.70 $\pm$ 7.73 a	0.36 $\pm$ 0.10 a
<i>Spodoptera eridania</i>						
Treatment	n	Larva (days)*	Pupae (days)*	Adult (days)*	Larva-to-adult period (day*)	Pupae weight (g)*
<i>Bt</i>	29	28.13 $\pm$ 3.12 a	11.93 $\pm$ 1.31 a	9.90 $\pm$ 2.72 a	49.97 $\pm$ 4.89 a	0.18 $\pm$ 0.03 a
non- <i>Bt</i>	26	31.69 $\pm$ 4.74 b	12.9 $\pm$ 2.16 a	12.87 $\pm$ 2.97 b	57.45 $\pm$ 6.8 b	0.17 $\pm$ 0.03 a

* Different letters indicate significant differences among treatments.

(Test: Kruskal Wallis, $\alpha \leq 0.05$). Pupal weight (Test: Tukey, $\alpha \leq 0.05$).

*Diferentes letras en las columnas indican diferencias significativas entre tratamientos (Prueba: Kruskal Wallis, $\alpha \leq 0,05$). Peso de pupa (Prueba: Tukey, $\alpha \leq 0,05$).

The larva-to-adult period for *S. cosmioides* was 44.31 days on *Bt* soybean and 46.70 days on non-*Bt* soybean, with no significant differences ($H= 0.48$; $p= 0.4881$). In contrast, *S. eridania* had a larva-to-adult period significantly longer on non-*Bt* soybean (57.45 days) compared to *Bt* soybean (49.97 days) ($H= 13.89$; $p= 0.0002$) (table 1, page 96).

Considering pupal weight, no significant differences were found for either species: *S. cosmioides* ($F= 0.95$; $p= 0.3359$) and *S. eridania* ($F= 3.77$; $p= 0.0577$) (table 1, page 96).

Larval feeding did not affect average number of eggs per female in either species. *S. cosmioides* had 3291.82 eggs on *Bt* soybean leaves and 3049.0 on non-*Bt* soybean leaves ($H= 0.08$; $p= 0.7782$). *S. eridania* had lower fecundity than *S. cosmioides*, with 841.79 eggs on *Bt* soybean and 830.70 eggs on non-*Bt* soybean ($H= 0.01$; $p= 0.9068$) (table 2). The Cry1Ac protein ingested by larvae did not affect fecundity in the studied species (table 2). Similarly, no significant differences were observed in fertility (percentage of hatched eggs) between treatment species ($H= 0.04$; $p= 0.8327$ for *S. cosmioides* and $H= 2.14$; $p= 0.1432$ for *S. eridania*) (table 2).

Table 2. Fecundity (number of eggs) and Fertility (% of hatched eggs) (Mean $\pm$ SD) of *Spodoptera cosmioides* and *S. eridania* fed *Bt* and non-*Bt* soybean leaves under controlled conditions.

Tabla 2. Fecundidad (número de huevos) y fertilidad (% de huevos eclosionados) (Media $\pm$ DE) de *Spodoptera cosmioides* y *S. eridania* alimentadas con hojas de soja *Bt* y no-*Bt* en condiciones controladas.

<i>Spodoptera cosmioides</i>			
Treatment	n	Number of eggs*	Hatched eggs (%) *
<i>Bt</i>	11	3291.82 $\pm$ 2089.99 a	50.44 $\pm$ 12.14 a
non- <i>Bt</i>	10	3049.0 $\pm$ 1855.57 a	52.08 $\pm$ 10.67 a
<i>Spodoptera eridania</i>			
Treatment	n	Number of eggs*	Hatched eggs (%) *
<i>Bt</i>	14	841.79 $\pm$ 391.13 a	70.75 $\pm$ 16,34 a
non- <i>Bt</i>	10	830.70 $\pm$ 356,73 a	60.85 $\pm$ 12,21 a

*Different letters in columns indicate significant differences between treatments. (Test: Kruskal Wallis $\alpha \leq 0.05$).

* Diferentes letras en las columnas indican diferencias significativas entre tratamientos (Prueba: Kruskal Wallis $\alpha \leq 0,05$).

Pre and post-oviposition periods were similar for both species under both larval feeding treatments (table 3, page 98), ($H= 1.32$; $p= 0.2395$ and $H= 0.85$; $p= 0.34$ for *S. cosmioides*, respectively; $H= 0.51$; $p= 0.4460$ and $H= 2.25$; $p= 0.1238$ for *S. eridania*, respectively). However, significant differences were found in the oviposition period for *S. eridania*, with 4.29 and 6.13 days in adults emerging from larvae fed *Bt* and non-*Bt* soybean leaves, respectively ($H= 4.62$; $p= 0.0293$).

Effect of *Bt* soybean on leaf consumption by *S. cosmioides* and *S. eridania*

Total leaf area consumption by *S. cosmioides* was lower when larvae were fed with *Bt* soybean ($T= -2.77$; $p= 0.0081$). In contrast, *S. eridania* showed no significant differences in leaf area consumption between *Bt* and non-*Bt* soybean leaves ($T= 0.05$; $p= 0.9585$) (table 4, page 98).

Table 3. Pre-oviposition, oviposition and post-oviposition periods of *Spodoptera cosmioides* and *S. eridania* (Mean $\pm$ SD) fed *Bt* and non-*Bt* soybean leaves under controlled conditions.**Tabla 3.** Períodos de preoviposición, oviposición y postoviposición de *Spodoptera cosmioides* y *S. eridania* (Media $\pm$ DE) alimentados con hojas de soja *Bt* y no *Bt* en condiciones controladas.

<i>Spodoptera cosmioides</i>				
Treatment	n	Pre oviposition (days)	Oviposition (days)	Post-oviposition (days)
<i>Bt</i>	11	4.18 $\pm$ 2.27 a	9.18 $\pm$ 3.12 a	1.09 $\pm$ 1.22 a
No <i>Bt</i>	10	2.91 $\pm$ 1.76 a	8.82 $\pm$ 2.93 a	1.55 $\pm$ 1.29 a
<i>Spodoptera eridania</i>				
Treatment	n	Pre-oviposition (days)	Oviposition (days)	Post-oviposition (days)
<i>Bt</i>	13	3.00 $\pm$ 1.24 a	4.29 $\pm$ 1.33 a	3.21 $\pm$ 1.19 a
No <i>Bt</i>	10	3.63 $\pm$ 1.85 a	6.13 $\pm$ 2.10 b	4.13 $\pm$ 1.46 a

* Different letters in columns indicate significant differences between treatments. (Test: Kruskal Wallis $\alpha \leq 0.05$).

* Diferentes letras en las columnas indican diferencias significativas entre tratamientos (Prueba: Kruskal Wallis $\alpha \leq 0,05$).

Table 4. Leaf area consumption of *Spodoptera cosmioides* and *S. eridania* (Mean $\pm$ SD) fed *Bt* and non-*Bt* soybean leaves under controlled conditions.**Tabla 4.** Consumo de área foliar de *Spodoptera cosmioides* y *S. eridania* (Media $\pm$ DE) alimentados con hojas de soja *Bt* y no *Bt* en condiciones controladas.

<i>Spodoptera cosmioides</i>		
Treatment	n	Consumption (cm ²) *
<i>Bt</i>	26	343.11 $\pm$ 19.79 a
non- <i>Bt</i>	23	398.93 $\pm$ 21.04 b
<i>Spodoptera eridania</i>		
Treatment	n	Consumption (cm ²) *
<i>Bt</i>	29	149.70 $\pm$ 4.45 a
non- <i>Bt</i>	26	150.00 $\pm$ 3.75 a

*Different letters in columns indicate significant differences between treatments. (Test: T, $\alpha \leq 0.05$).

* Diferentes letras en las columnas indican diferencias significativas entre tratamientos. (Test: T, $\alpha \leq 0,05$).

DISCUSSION

gM crops expressing Cry proteins are crucial for pest control. Besides killing susceptible species, these crops can have sublethal effects on tolerant species, through direct or indirect exposure, leading to broader ecological changes (40). *Spodoptera cosmioides* and *S. eridania* exhibit tolerance against the Cry1Ac protein (2) due to the type and quantity of receptor proteins in larval midgut membranes, low receptor affinity, or rapid protein degradation (35). Thus, insect exposure to stress factors like Cry1Ac protein expressed by *Bt* soybean may enhance fitness of the exposed population (17, 18). This explains why *S. eridania* individuals exhibited shorter durations in both larval and adult stages, and a reduced larva-to-adult period when fed soybean leaves expressing the Cry1Ac protein. In contrast, *S. cosmioides* only experienced a decrease in the pupal period when fed on insect-resistant GM soybeans (*Bt*).

Regarding larval cycle, our results for *S. cosmioides* agree with Bernardi *et al.* (2014) and Silva *et al.* (2019), who observed a similar duration for the last larval stage under the same treatments. In contrast, *S. eridania* showed significant differences between treatments with an average duration of 28.13 days on *Bt* soybeans and 31.69 days on non-*Bt* soybeans. These results are consistent with those reported by Bortolotto *et al.* (2014) and Rabelo *et al.* (2020), who observed a significant reduction of 2 days in the larval stage of *S. eridania* when fed GM soybeans expressing Cry1Ac.

Our results showed that *S. eridania* adults from *Bt* soybeans live 3 days less than those from the control group. In contrast, Silva (2013) and Bortolotto *et al.* (2014) reported a significant 3 days-increase in longevity of *S. eridania* males when reared on *Bt* soybean leaves. This discrepancy suggests that Cry1Ac might induce asynchronous adults' emergence between the two cultivars, potentially reducing mating chances in natural conditions. According to Jakka *et al.* (2014) and Murúa *et al.* (2019), the non-simultaneous emergence of adults in both cultivars could compromise the refuge strategy to avoid or delay resistance emergence. On the other hand, we found a shortened life cycle of *S. eridania* (7.48 days) when larvae were fed soybeans expressing Cry1Ac, as seen by Ramírez & Gómez (2010), who reported an average life cycle of 51.72 days for *S. eridania* with artificial diet.

Several studies have demonstrated that noctuid pupae weight can vary with temperature, host plants, and exposure to sublethal insecticide concentrations or *Bt* crop toxins (22). However, our results indicate that *Bt* protein did not affect pupal weight of either species. Additionally, feeding larvae with *Bt* soybean leaves did not affect the reproductive capacity of either *Spodoptera* species, as observed by Silva *et al.* (2016) and Sosa *et al.* (2020), in *S. cosmioides* for larvae fed with *Bt* soybean leaves. However, Páez Jerez *et al.* (2022) reported more eggs per female in *S. cosmioides* individuals fed *Bt* soybean. According to Specht & Roque-Specht (2019), fecundity in *S. cosmioides* is highly variable, with females capable of producing up to 5000 eggs/female, higher than for *S. eridania*, *S. albula*, *S. frugiperda* and *S. littoralis* (26, 27). In our study, average egg number per *S. eridania* female is consistent with Silva (2013), who reported similar fecundity in females reared on both *Bt* and non-*Bt* soybeans during larval stage, with averages of 881.35 and 911.85 eggs per female, respectively.

We found that exposure to the insecticidal protein Cry1Ac during larval stage shortened the oviposition period in *S. eridania*. Although the literature lacks specific data on the oviposition period of *S. eridania* fed on *Bt* cultivars, previous studies have reported variable oviposition periods ranging from 4.2 days to 6.75 days when larvae were reared on non-*Bt* soybean leaves (14).

Biological fitness is the ability of an organism to compete successfully, pass on its genes to subsequent generations and influence population density and the potential to become a pest. However, insecticide exposure can have variable effects, enhancing or reducing performance, potentially leading to adverse impacts on survival, developmental rate, reproduction, and adult longevity (3). This phenomenon has been documented in several Lepidoptera species exposed to *Bt* protein (16). In our study, we observed a reduced pupal period in *S. cosmioides* and shortened larval, adult, and larval-to-adult cycles and oviposition periods in *S. eridania* when fed *Bt* soybean leaves.

Food quantity and quality directly influence host plant preference affecting biological, physiological, and behavioral features (11). While some studies have found no effects of Cry toxins on foliar consumption in lepidopterans (11), other research reports less leaf consumption due to Cry proteins in corn (5), as we found for *S. cosmioides*. According to Zurbrugg *et al.* (2010), glyphosate-resistant soybeans expressing the Cry1Ac toxin have more carbohydrates and lower protein content than non-transgenic cultivars. This variation in nutritional composition may influence insect food preference as seen in *S. cosmioides* when fed on Cry1Ac-expressing soybean.

CONCLUSION

Transgenic crops expressing Cry insecticidal proteins are valuable tools for controlling susceptible pests. However, they may also induce changes in life cycles, population dynamics, reproductive stages, feeding behavior, or longevity of non-target species. Understanding developmental and reproductive parameters of these non-target pests is essential for predicting population growth and species dynamics within agricultural systems. Our findings shed light on the biology of *S. cosmioides* and *S. eridania* in Bt soybean crops in Argentina, considering foliar consumption and herbivorous capacity. Since our experiments were conducted under controlled conditions, these investigations should further assess actual field damage caused by *Spodoptera* species in soybean crops.

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