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Biological parameters of *Spodoptera cosmioides* Walker (Lepidoptera: Noctuidae) in cotton varieties*

Parâmetros biológicos de **Spodoptera cosmioides** *Walker (Lepidoptera: Noctuidae) em variedades de algodoeiro*

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ABSTRACT: *Spodoptera cosmioides* Walker is a polyphagous insecticide-resistant species found in various regions of Brazil. Despite occurring at low densities, it is considered a potential pest of cotton, soybean, and bean crops in the Brazilian *cerrado*. This work investigated the comparative biology of *S. cosmioides* in the commercial transgenic cotton NuOPAL (Bollgard I, Evento 531) and the conventional isoline variety DeltaOPAL. The experiment was conducted under laboratory conditions (26 ± 1°C; relative humidity: 70 ± 10%; photoperiod: 12h), using newly-hatched and individualized larvae. We evaluated the duration and viability of immature stages, duration of the biological cycle, pupal weight, sex ratio, percentage of deformed adults and of adults trapped in the pupal case, adult longevity, fecundity, and egg viability. The biological parameters observed showed no significant differences between the two varieties studied, which enabled high consumption, high assimilation, high fecundity, and viability for *S. cosmioides*.

KEYWORDS: *Gossypium hirsutum*; transgenic plants; Bollgard; CryIAc.

RESUMO: *Spodoptera cosmioides* Walker é uma espécie polífaga, resistente a inseticidas em várias regiões do país e, apesar de ocorrer em baixas densidades, é considerada uma praga potencial para as culturas de algodão, soja e feijão no cerrado. Neste trabalho, estudou-se a biologia comparada de *S. cosmioides* sobre o cultivar transgênico comercial de algodoeiro NuOPAL (Bollgard I, Evento 531) e sobre o cultivar isolinha convencional DeltaOPAL. O experimento foi desenvolvido em condições de laboratório (26 ± 1°C; UR: 70 ± 10%; fotofase: 12h) a partir de lagartas recém-eclodidas e individualizadas. Foram avaliados a duração e a viabilidade das fases imaturas, a duração do ciclo biológico, o peso de pupas, a razão sexual, a porcentagem de deformação de adultos e de adultos não liberados dos invólucros pupais, a longevidade de adultos, a fecundidade e viabilidade de ovos. Os parâmetros biológicos observados não diferiram significativamente em relação às duas cultivares, que proporcionaram alto consumo, alta assimilação, alta fecundidade e viabilidade à *S. cosmioides*.

PALAVRAS-CHAVE: *Gossypium hirsutum*; planta transgênica; Bollgard; CryIAc.

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INTRODUCTION

Cotton production in Brazil has increased considerably with the advancement of technology, allowing the country to move from being the world's largest importer to the second largest exporter in 12 years (ABRAPA, 2016; SEVERINO et al. 2019). With an average production volume close to 2.7 million tons (CONAB, 2019), the country ranks among the top five world producers alongside countries like India, China, Pakistan, and the USA (SEVERINO et al. 2019).

Insect pests are an important agronomic problem in this crop, causing great economic damage annually. The use of chemical products for pest control can reach up to 25% of the production cost (EMBRAPA MEIO AMBIENTE, 2016).

The availability of genetically modified cotton cultivars represented a significant advance in pest control in the last two decades (SANTOS; TORRES, 2010). Genetically modified plants increase production (BETZ et al., 2000), lower levels of mycotoxins (DOWD, 2000), and reduce the use of insecticides (ROMEIS et al., 2006) while favoring the maintenance of natural enemies (GOULD, 1998), in addition to helping in pest control and contributing to delay the evolution of resistance (MASCARENHAS; LUTTRELL, 1997). Therefore, the use of genetically modified cultivars is considered a tactic compatible with the Integrated Pest Management of cotton crops.

Genetically modified plants with the genes of *Bacillus thuringiensis* var. *kurstaki* (Bt) express toxic proteins (alpha-endotoxins) during sporulation, which have a lethal effect when ingested by Diptera and Lepidoptera larvae (SCHNEPF et al., 1998). The first commercial transgenic cotton (Bt) cultivar, known as Bollgard or Bollgard I, was produced in 1989 and commercially released in the USA and Australia in 1996/97. In Brazil, its release occurred in 2005 (CTNBio, 2005). Until 2013, the planting of transgenic cotton cultivars in Brazil corresponded to 47% of the planted area (GRAVINA, 2013). Brazil currently has about 940 thousand ha planted with transgenic cotton (ISAAA, 2017).

Bollgard I (NuOPAL) cotton expresses the Cry1Ac protein, which provides resistance to some caterpillars that attack cotton crops in Brazil: *Heliothis virescens* (tobacco budworm), *Pectinophora gossypiella* (pink bollworm), and *Alabama argillacea* (cotton leafworm) (BARROSO; HOFFMANN, 2007). Currently, Bollgard II cotton is available in Brazil and expresses two Bt proteins, Cry1Ac and Cry2Ab2, both specific and toxic to some caterpillars.

Cry1Ac toxicity is not effective in suppressing *Spodoptera frugiperda* and *S. eridania* (ADAMCZYK et al., 1998), increasing the tolerance of these species to the consumption of this cultivar (ADAMCZYK; SUMERFORD, 2001). Consequently, outbreaks of these pest species in Bollgard cotton require applications of foliar insecticides to keep these populations below the level of economic damage (HOOD, 1997; SMITH, 1997).

The joint expression of Cry1Ac and Cry2Ab2 proteins (Bollgard II) suppresses populations of the *Spodoptera* and *Helicoverpa* species (MONSANTO, 2016). Different pest species present distinct responses to the use of Bt technologies since mortality varies according to the target species and the expressed proteins. ADAMCZYK et al. (2008) evaluated the Bollgard I, Bollgard II, and WideStricke technologies regarding the mortality of *S. frugiperda* and *S. eridania* and reported that the different species presented distinct responses to the cultivars.

Spodoptera cosmioides has been reported as a species with high damage potential for several important crops due to its high polyphagy and voracity (QUINTELA et al., 2007; FARIA, 2010; LINK, 2010). Despite its high degree of polyphagy, it is considered a pest in some crops, especially in those that use excessively broad-spectrum insecticides (HABIB et al., 1982). *S. cosmioides,* together with *S. eridania*, form the main group of caterpillars that attack soybean pods, assuming importance from the beginning of the reproductive phase of this crop (GAZZONI; YORINORI, 1995). In addition, in the states of São Paulo, Paraná, and Santa Catarina, outbreaks of this insect caused severe damage to crops of economic interest, such as cotton and soy (HABIB et al., 1982), apple (NORA; REIS FILHO, 1988), and onion (BAVARESCO et al., 2003).

PORTILLO et al. (1991; 1996) associated the most significant damages of this insect to corn crops cultivated in areas that adopted weed chemical control, since these weeds were used as food by caterpillars. LEITE et al. (2008) reported a strong outbreak of *S. cosmioides* in the 2007 sunflower harvest, leading to the loss of significant areas of this crop in Goiás, mainly in the south of the state.

Despite its potential as a pest of various important crops, knowledge about the *S. cosmioides* biology in cotton cultivars is still insufficient. With the introduction of new cultivars and the increased adoption of crops with transgenic varieties, it is essential to know their influence on the biological cycle of the insect, mainly to provide information about larval survival or viability and monitor the emergence of populations resistant to these plants.

The knowledge of insect biology is fundamental for the development of effective management strategies, following the Integrated Pest Management (PARRA, 2000). Thus, the present study aimed to compare biological aspects of *S. cosmioides* in the transgenic cotton cultivar NuOPAL (Bollgard I) and the non-genetically modified isoline cultivar DeltaOPAL.

MATERIAL AND METHODS

This research was carried out at the Biological Control Laboratory of the Phytosanitary Department of the School of Agricultural and Veterinary Studies (Faculdade de Ciências

Agrárias e Veterinárias — FCAV) of Universidade Estadual Paulista (UNESP), Jaboticabal campus, using refrigerated incubators (BOD) maintained at a temperature of 25 ± 1°C, 70 ± 10% relative humidity, and 12 hours of photoperiod in all laboratory tests.

The commercial cultivars used in this work were DeltaOPAL and NuOPAL. The leaves used to feed the caterpillars during the experiments were obtained from cultivars planted in a field following agronomic recommendations normally used in cotton cultivation.

Rearing S. cosmioides in laboratory conditions

S. cosmioides caterpillars were collected in cotton cultivar DeltaPine Acala 90, established in the experimental area of UNESP, Jaboticabal campus. The insects were placed in plastic containers (12 cm diameter × 9 cm height) with their base covered with filter paper and the top closed with a voile cover to provide aeration and prevent the harmful proliferation of microorganisms. The caterpillars fed on cotton leaves from the cultivar DeltaPine Acala 90, collected from the upper third of the plants, washed in a 0.15% sodium hypochlorite solution and then with abundant water. The choice of cultivar aimed to avoid possible pre-imaginal conditioning in the subsequent tests with the other cultivars (Bt cultivar and its isoline). The leaves were changed according to the consumption by the caterpillars so that there was no lack of food.

Pupae were separated by sex, according to BUTT; CANTU (1962). Ten newly hatched females and males were placed in cylindrical PVC cages (20 cm height × 19.5 cm diameter) with the base sealed with polyethylene discs (21.5 cm diameter) and the top covered with voile fixed by elastics to promote the emergence and reproduction of adults. These cages were internally covered with white paper, where the females laid eggs.

The adults were fed a 10% honey solution absorbed in hydrophilic cotton placed on a plastic lid (4.5 cm diameter) at the bottom of the cage.

Papers with eggs were removed from the cages daily, and the eggs were placed in a plastic container covered with voile. After the caterpillars hatched, cotton leaves from the cultivar DeltaPine Acala 90 were supplied, as previously mentioned.

Biological parameters of S. cosmioides fed with conventional and transgenic cotton leaves

We used a completely randomized design (CRD) with two treatments consisting of the cotton genotypes DeltaOpal and NuOpal (Bollgard I), each with 40 repetitions (neonate caterpillars) to assess the duration and viability of immature stages and 10 repetitions (10 couples individualized in cages) for parameters related to the adult phase, such as longevity and reproduction.

Insects from the F2 generation of laboratory rearing were used to evaluate the biological parameters of the larval phase. After hatching, the caterpillars were separated in Petri dishes lined with filter paper and kept on leaves of the cultivars analyzed, with this food always available. These Petri dishes were cleaned daily with the removal of feces and changing of the filter paper.

The pupae were weighed and separated by sex (according to BUTT; CANTU, 1962) 24 hours after pupation and transferred to plastic containers (7.5 cm diameter × 5 cm height) where they remained until emerging as adults. The sex ratio was obtained using the formula proposed by SILVEIRA NETO et al. (1976).

To analyze the biological parameters of adults, each repetition consisted of placing a couple of *S. cosmioides* in a cylindrical PVC cage (20 cm height × 19.5 diameter) lined with white paper (for egg-laying), with the base sealed with polyethylene discs (21.5 cm diameter) lined with filter paper and the top covered with voile fixed with elastics.

We evaluated the duration and viability of immature stages, duration of the life cycle, pupal weight, sex ratio, percentage of deformed adults and of adults trapped in the pupal case, adult longevity, fecundity, and egg viability.

The data gathered from the different biological parameters were analyzed by the F test (ANOVA), and the treatment means were compared using the Tukey test at a 5% probability level in the ASSISTAT 7.7 software.

RESULTS AND DISCUSSION

The mean duration of the larval phase was approximately 25 days in both cultivars (Table 1), with 100% viability in caterpillars fed with leaves of the cultivar NuOPAL and 90% in those fed with DeltaOPAL (Table 2). HABIB et al. (1982) identified a shorter duration of the larval phase of *S. cosmioides* reared in the cultivar IAC-20 – 13.4 days to complete the phase in the temperature range of 22.3 – 30.4°C, with 95% viability. SARRO (2006) studied the biology of *Spodoptera frugiperda* in cotton and detected a larval phase of 22 days in the cultivar DeltaOPAL, with 100% viability.

BAVARESCO et al. (2003) found a significant difference in the duration of the larval phase of *S. cosmioides* among different host plants — 20.2 days for castor oil plant, 22.3 days for onion, and 28 days for soybean, with viability of 77.3, 74.0, and 45.3%, respectively. The results obtained in this work and those cited in the literature demonstrate that caterpillars of the genus *Spodoptera*, especially *S. cosmioides*, develop better by feeding on cotton leaves than on soybean leaves, which is a crop frequently associated with this species.

The average duration of the pre-pupa phase was two days in both cultivars, with 100% viability (Table 1). This value is higher than that found for *S. cosmioides* in the cultivar IAC-20, with 1.3 and 1.4 days for males and females, respectively (HABIB et al., 1982). Working with *S. frugiperda*, SARRO (2006) obtained a similar result — 2.3 days for the pre-pupal phase in the DeltaOPAL cultivar, with 100% viability.

The pupal phase lasted, on average, 13.6 days in the cultivar DeltaOPAL and 13.1 days in NuOPAL, with 69.4 and 77.5% viability, respectively (Table 1). HABIB et al. (1982) noted a shorter duration (9.5 days) in the cultivar IAC-20, while SARRO (2006) identified a mean duration of 10 days for the pupal phase of *S. frugiperda* in the DeltaOPAL cultivar, with 92% viability.

The average pupal weight in both cultivars was approximately 450 mg (Table 1). This result is much higher than that found by HABIB et al. (1982), who detected an average weight of 249 mg in the cultivar IAC-20. SARRO (2006) reported low average weight, around 184 mg, for *S. frugiperda* fed with the cultivar DeltaOPAL. The high viability in the larva and pupa phases and the expressive weight gain in the pupa phase indicate that the cultivars analyzed are well accepted for consumption by the caterpillars and have high assimilation in the immature stages of *S. cosmioides*.

The duration of the *S. cosmioides* larva–adult period was approximately 40 days in the two cultivars analyzed (Table 1).

The sex ratio was 0.40 in the cultivar DeltaOPAL and 0.45 in NuOPAL, not affecting the proportion of emergence between sexes, which was around 1:1 in the studied cultivars.

As for adult longevity, females were more long-lived than males, presenting a mean of approximately 8.5 days, 2 days longer than males (Table 2). HABIB et al. (1982) identified higher longevity (13.2 days) when the caterpillars fed on leaves of the cultivar IAC-20.

Total fecundity varied between 1541.1 and 1910.7 eggs in the cultivars DeltaOPAL and NuOPAL, respectively (Table 2), although these results did not differ statistically at the 5% probability level. HABIB et al. (1982) reported a lower total reproductive capacity for *S. cosmioides*, with 1309 eggs per

Table 1. Mean ± standard error of the mean (days) and viability (%) of the duration of the larva, pre-pupa, and pupa stages, larva–adult period, and pupal weight of *Spodoptera cosmioides* fed with transgenic cotton cultivar NuOPAL (Bollgard I) and its non-transgenic isoline DeltaOPAL. Temperature: 26 ± 1°C; relative humidity: 70 ± 10%; photoperiod: 12h. Jaboticabal, São Paulo, 2008.

Cultivars	Biological parameters of immature stages							
	LARVA		PRE-PUPA		PUPA		LARVA–ADULT	
	Duration (days)	Viability (%)	Duration (days)	Viability (%)	Duration (days)	Viability (%)	Duration (days)	Viability (%)
DeltaOPAL	25.14 ± 0.50a	90	2.1 ± 0.10a	100	13.6 ± 0.24a	69.4	40.6 ± 0.56a	62.5
NuOPAL	25.12 ± 0.38a	100	2.3 ± 0.19a	100	13.1 ± 0.26a	77.5	40.2 ± 0.52a	77.5

Mean values followed by the same letter in the column do not differ statistically from each other according to the Tukey test at a 5% probability.

Table 2. Mean ± standard error of the mean of adult longevity, duration of pre-reproductive, reproductive, and post-reproductive periods (in days), total fecundity (number of eggs/female), duration of one generation, percentages of deformed adults and of adults trapped in the pupal case, and number of viable eggs of *Spodoptera cosmioides* fed with transgenic cotton cultivar and its non-transgenic isoline. Temperature: 26 ± 1°C; relative humidity: 70 ± 10%; photoperiod: 12h. Jaboticabal, São Paulo, 2008.

Biological parameters of adults	Cultivars	
	DeltaOPAL	NuOPAL (Bollgard I)
Longevity of males	6.1 ± 0.56a	5.6 ± 0.65a
Longevity of females	8.8 ± 0.51a	8.3 ± 0.97a
Pre-reproductive period	3.0 ± 0.25a	3.7 ± 0.33a
Reproductive period	4.7 ± 0.51a	3.7 ± 0.47a
Post-reproductive period	1.1 ± 0.27a	1.2 ± 0.46a
Total fecundity	1541.1 ± 213.96a	1910.7 ± 318.28a
Viable eggs (%)	72.9 ± 5.16a	54.4 ± 9.21a
Generation time	47.1 ± 0.72a	46.9 ± 0.60a
Deformed adults	16%	29%
Adults trapped in the pupal case	8%	12.9%

Mean values followed by the same letter in the column do not differ statistically from each other according to the Tukey test at a 5% probability.

female, on average, in the cotton cultivar IAC-20. In a study on lepidopteran biology, JOHANSSON (1964) stated that feeding different cultivars of the same plant species to the insects can influence egg-laying. Also, according to this author, the variation in the amount of food consumed or even physical or chemical differences in the food are responsible for these effects on reproduction.

The cotton cultivar NuOPAL presented the highest percentage of deformed adults (29%) and adults trapped in the pupal case (13%) (Table 2). This finding demonstrates that, although the caterpillars fed heavily on this cultivar, this consumption probably led to nutritional deficiencies or compounds harmful to the development of *S. cosmioides*. LARA (1991) points out that nutritional inadequacy generally influences the reproductive capacity of females, resulting in reduced fertility, in addition to affecting the mortality rate in the metamorphosis to the adult phase. New studies should be conducted to investigate the action of the cultivar NuOPAL on the physiology of the adult *S. cosmioides*.

CONCLUSIONS

The results obtained for all parameters analyzed in the two cultivars did not show a statistically significant difference at the 5% probability level. Thus, we can conclude that the consumption of the NuOPAL and DeltaOPAL cultivars does not harm the survival and reproduction of this insect.

The NuOPAL and DeltaOPAL cotton cultivars fed to the caterpillars acted similarly on the biology of *S. cosmioides*, providing high viability, high fecundity, and similar duration of life stages. In addition, leaf consumption of both cultivars was high during the larval phase. As this is the stage in which *S. cosmioides* damages the cotton crop, outbreaks of this insect would justify the use of foliar insecticides, which would not be desirable from an economic point of view. Furthermore, the inefficacy of the Cry1Ac endotoxin expressed by NuOPAL (Bollgard I) in suppressing the larval phase of *S. cosmioides* may select this species, which, on a longer-term, could become more prevalent in crops since they have several hosts widely grown during the year and could migrate between them.

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Availability of data and material: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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