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Multivariate Approach Helps in Understanding the Tritrophic Interaction of Bt cotton, *Aphis gossypii* and *Chrysoperla externa*

Abordagem Multivariada Auxilia no Entendimento da Interação Tritrófica do Algodão Bt, *Aphis gossypii* e *Chrysoperla externa*

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Abstract

In cotton, *Chrysoperla externa* stands out for its ability to prey on arthropods. The aim of this study was to evaluate the reproductive aspects and longevity of *Chrysoperla externa* when the larvae were fed *Aphis gossypii* from transgenic (*Bt*) and conventional cotton. Nine cotton cultivars were used: Conventional FM993, DP604 BG (Bollgard I®-Cry1Ac), NuOpal (Bollgard I®-Cry1Ac), DP90 B (Bollgard I®-Cry1Ac), Acala DTL90B (Bollgard I®-Cry1Ac), DP555 BGRR (Bollgard I®-Cry1Ac), DP1228 B2RF (Bollgard II®-Cry1Ac+Cry2Ab2), PHY440 W (WideStrike®-Cry1Ac+Cry1F) and FM975 WS (WideStrike®-Cry1Ac+Cry1F). The adults of *C. externa* from the F_5 generation, in which the larvae were fed *A. gossypii* from the cotton cultivars, were sexed and kept as a pair per cage. Longevity and reproductive potential were assessed daily (preoviposition, oviposition and postoviposition periods; daily and total oviposition capacity; percentage of eggs without pedicels; and egg viability). Principal component (PC) and cluster analyses were performed using the all measured variables. All variables showed that there is variation in the response of C. externa fed during the immature stages with *A. gossypii* from cotton cultivars with different Bt technologies. *C. externa* larvae that fed on *A. gossypii* from transgenic plants presented a reduction in reproductive potential and adult longevity. The PC and the formed 3 groups for the CA analyses,

mostred the reproductive aspects and longevity of *C. externa* were negatively influenced when the larvae were fed *A. gossypii* from the cultivars DP90 B and DP555 BGRR, which express the Cry1Ac protein and affect genetic characteristics when the larvae are fed the nontarget pest.

Keywords: Chrysopids. Cotton Aphid. Reproductive Potential. Transgenic Cotton.

Resumo

No algodão, *Chrysoperla externa* se destaca pela sua capacidade de predar artrópodes. O objetivo deste estudo foi avaliar os aspectos reprodutivos e a longevidade de *Chrysoperla externa* quando as larvas foram alimentadas com *Aphis gossypii* de algodão transgênico (*Bt*) e convencional. Foram utilizadas nove cultivares de algodão: Convencional FM993, DP604 BG (Bollgard I®-Cry1Ac), NuOpal (Bollgard I®-Cry1Ac), DP90 B (Bollgard I®-Cry1Ac), Acala DTL90B (Bollgard I®-Cry1Ac), DP555 BGRR (Bollgard I®-Cry1Ac), DP1228 B2RF (Bollgard II®-Cry1Ac+Cry2Ab2), PHY440 W (WideStrike®-Cry1Ac+Cry1F) e FM975 WS (WideStrike®-Cry1Ac+Cry1F). Os adultos de *C. externa* da geração F_5, em que as larvas foram alimentadas com *A. gossypii* das cultivares de algodão, foram sexados e mantidos como um par por gaiola. A longevidade e o potencial reprodutivo foram avaliados diariamente (períodos de pré-oviposição, oviposição e pós-oviposição; capacidade de oviposição diária e total; porcentagem de ovos sem pedicelos; e viabilidade dos ovos). Análises de componentes principais (CP) e de agrupamento foram realizadas usando todas as variáveis medidas. Todas as variáveis mostraram que há variação na resposta de *C. externa* alimentada durante os estágios imaturos com *A. gossypii* de cultivares de algodão com diferentes tecnologias Bt. Larvas de C. externa que se alimentaram de *A. gossypii* de plantas transgênicas apresentaram redução no potencial reprodutivo e na longevidade adulta. A CP e os 3 grupos formados para as análises de CA, a maioria dos aspectos reprodutivos e a longevidade de C. externa foram influenciados negativamente quando as larvas foram alimentadas com *A. gossypii* das cultivares DP90 B e DP555 BGRR, que expressam a proteína Cry1Ac e afetam as características genéticas quando as larvas são alimentadas com a praga não alvo.

Palavras-chave: Crisopídeos. Pulgão do Algodão. Potencial Reprodutivo. Algodão Transgênico.

1 Introduction

Among the natural enemies of the cotton crop *Chrysoperla externa*, Hagen, 1861 (Neuroptera: Chrysopidae) stands out for its ability to prey on various arthropod pests, such as mealybugs (Bezerra *et al.,* 2006; Bonani *et al.,* 2009), aphids (Figueira; Lara, 2004; Schlick-Souza *et al.,* 2011; Soffiantini Lira; De Luna Batista, 2006), whiteflies (Auad *et al.,* 2001; Castro *et al.,* 2016; Castro-Lopez; Martinez-Osorio, 2016), mites (Morando *et al.,* 2014; Vilela *et al.,* 2009), thrips (Bastidas; Devia; Amaya, 2010; Dos Santos; Souza; Hernandez, 2024), and eggs and neonate larvae of various lepidopteran species (Carvalho; Souza, 2000).

The larvae of *C. externa* have high search capacity, voracity, and reproductive potential and are easy to rear under laboratory conditions (Carvalho; Souza, 2000; Fonseca *et al.,* 2000; Murata *et al.,* 2006; Bezerra *et al.,* 2009), which has facilitated their use as a biological control (Carvalho *et al.,* 2023).

Lacewings are polyphagous predators; however, they have a certain preference for aphids (Ramalho *et al.,* 2014; Schlick-Souza *et al.,* 2011). Several species only predate on the larval stage,

requiring substances rich in proteins and carbohydrates in their diet (Murata *et al.,* 1996). The quality of the prey consumed during the larval stage can influence the viability of predator species. According to Panizzi, Parra and Silva (2012) and Pinto *et al*. (2019); the quantity and quality of the food eaten by larvae can influence aspects of biological development; reproductive capacity; longevity and adult competition.

Studies on the impact of Bt plants on tritrophic interactions have been carried out on nontarget pests and their predators (Mota *et al.,* 2012). The authors reported that the biology and development of *C. externa* larvae that fed on aphids reared on *Gossypium* Bt leaves were not affected by the Cry1Ac toxin. Shera, Karmakar and Sharma (2018) reported that transgenic cotton expressing single (Cry1Ac) or double (Cry1Ac and Cry2Ab) toxins manifested no apparent effect on the fitness of the predator [*Chrysoperla zastrowi sillemi* (Esben-Petersen)] (Neuroptera: Chrysopidae) through its prey *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae)*, Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and *Amrasca biguttula biguttulla* (Ishida) (Hemiptera: Cicadellidae).

The increase in the protection of Bt technology is related to the inclusion of new toxins in plants and, with this, a greater spectrum of protection, which is the main component for positioning cultivars for deployment in the field. This is why ongoing studies into the impact of cultivars with Bt proteins on nontarget insects and their food chain are necessary.

In tritrophic interactions, plants can interact indirectly with lacewings, which is associated with the chemical composition of the host plants on which the prey is fed. Prey food, in turn, can have greater or lesser nutritional value, as well as toxicity. Parrott (1990) reported the presence and content of secondary components such as gossypol, which can affect predator populations by reducing development and reproduction rates and increasing mortality (Albuquerque, 2009). In addition to gossypol, cotton may contain five other terpenoid aldehydes that make cotton resistant to herbivory (Meyer; Vorster; Dubery, 2004).

It is important to carry out studies evaluating the impacts of tritrophic interactions, transgenic cotton and the presence of secondary components in the diet of prey offered to lacewings, given that the food consumed during the larval stage can influence the biological development and reproductive capacity of this predator, since natural mortality caused by natural enemies is of paramount importance.

The aim of this study was to evaluate the reproductive aspects and longevity of *Chrysoperla externa* when larvae were fed *Aphis gossypii* from cotton with different Bt proteins.

2 Material and Methods

The experiment was conducted in the Entomology Laboratory at the University Unit of Cassilândia of Universidade Estadual de Mato Grosso do Sul (UEMS) in an air-conditioned room at

25± 1 °C and an RH of 70%± 10%.

The experiment used newly emerged adults from the F5 generation of *Chrysoperla externa* whose immature stages were fed on *Aphis gossypii* fed nine cotton cultivars [1-Conventional FM 993 (control) and the following genetically modified cultivars: 2- DP 604 BG, 3- NuOpal, 4- DP 90 B, 5- Acala DTL 90B, 6- DP 555 BGRR (Bollgard I® technology - Cry1Ac); 7- DP 1228 B2RF (Bollgard II® technology - Cry1Ac+Cry2Ab2); 8- PHY 440 W and 9- FM 975 WS (WideStrike® technology - Cry1Ac+Cry1F)].

After the biological development of *C. externa*, newly emerged adults were separated by sex by observing the external genitalia under a stereoscope, forming couples with the same diet during the respective larval stages. Each pair was placed in PVC cages, 23 cm high and 10 cm in diameter, lined with white sulfite paper as a substrate for oviposition, with the upper end sealed with voil fabric and the lower end resting on a PVC tray lined with paper towels (Carvalho; Souza, 2000).

The diet consisting of brewer's yeast and honey was provided at a 1:1 ratio; according to Ribeiro (1988), this ratio is adequate for the development of the reproductive stages. Distilled water soaked in absorbent cotton was provided in a plastic container. Both the diet and the water were replaced every three days (Soares; Macedo, 2000; Soares; Almeida, 2001).

Reproductive aspects were assessed: preoviposition (POP), oviposition (OP) and postoviposition (POPo) periods; daily oviposition capacity (DOC); total oviposition capacity per female (TOC); percentage of eggs without pedicels (EWP); egg viability (EV); and longevity of males (LM) and females (LF).

To assess the preoviposition period, it was quantified in days, from the emergence of the adult until the first oviposition of each female. After the first egg was laid, the eggs were collected every 24 hours via scissors and a brush, and the daily oviposition/female ratio was quantified (Figueira; Lara; Cruz, 2002). The total oviposition capacity was averaged from the daily egg/female values. Every day, the counted eggs were individually placed in glass tubes (2.5 × 8.5 cm), and the number of eggs without pedicels was quantified. To assess egg viability, the neonate larvae from the individual eggs were counted. For the longevity parameter, the daily mortality of females and males was assessed, in which the larval stages received aphids from Bt cultivars as a food source.

The experimental design used was entirely randomized (DIC) with nine treatments (cotton cultivars where the aphids were reared to feed the predator) and four replications (each replication consisted of 20 cages containing a pair of predators). The data were subjected to analysis of variance, and the means were compared via the F test. When differences were detected, the means were compared via the Tukey test at 5% probability via the RBio program version 166 (Bhering, 2017). Principal component analysis was carried out using all the variables and grouping the cultivars a priori while considering the presence of Bt proteins. Dissimilarity and clustering

analyses were carried out using the average values of the variables evaluated to calculate the Euclidean distance, and with these values, clustering was carried out via the UPGMA (unweighted pair group method with arithmetic mean) method to better differentiate the cultivars and detect the variables that most contribute to the diversity between them.

3 Results and Discussion

According to the ANOVA results (Table 1), all the variables evaluated were significantly different at the 0.01% probability according to the ANOVA F test. This result shows that there is variation in the behavior of *C. externa* adults when their larval stage is fed *A. gossypii* from Bt cotton cultivars with different protein combinations (Table 1). Adequate CVs were obtained for all the variables, with values of less than 20%, demonstrating the accuracy of the data obtained. Compared with those of the control (FM 993), the adults of *C. externa* fed during the larval stage with *A. gossypii* from the genetically modified cultivars presented a shorter preoviposition period, with the exception of NuOpal and DP 90 B, both of which contain the Cry1Ac protein (Table 1).

Table 1 - Variables obtained when evaluating *C. externa* fed during the immature stages with *A. gossypii* from cotton cultivars with different Bt technologies. The temperature was 25 ± 1°C, the RH was 70 ± 10%, and the photoperiod was 12 hours

Cultivars	Proteins	POP[ŧ] (days)	OP (days)	POPo (days)	DOC (egg days^{-1})	TOC (egg days^{-1})	EWP (%)	EV (%)	LF (days)	LM (days)
CONV. FM 993	-	6.92a	41.08a	3.16bc	21.08a	597.50a	9.12d	93.38a	51.25a	60.38a
DP 604 BG	Cry1Ac	6.41b	38.98a	3.00c	20.42ab	597.50a	10.33cd	90.51a	51.16a	59.91a
NUOPAL	Cry1Ac	7.00a	38.91a	3.23bc	18.90abc	590.59a	12.09cd	88.13ab	50. 97a	60.16a
DP 90 B	Cry1Ac	7.00a	26.00c	3.00c	8.75e	234.75e	30.75a	49.02d	38.00c	48.50c
ACALA DTL 90B	Cry1Ac	6.16bc	31.92b	3.16bc	17.96bc	563.63ab	14.96c	85.35b	43. 58b	52.08b
DP 555 BGRR	Cry1Ac	6.00c	24.00c	3.75ab	10.5e	295.50d	27.75a	53.06d	36.75c	46.75c
DP 1228 B2RF	Cry1Ac+Cry2Ab	6.08bc	31.75b	3.83ab	16.75cd	503.00c	20.62b	59.12cd	44.16b	54.58b
PHY 440 W	Cry1Ac+Cry1F	6.37b	32.00b	4.00a	19.25abc	525.37bc	15.43bc	70.54bc	43.75b	55.62b
FM 975 WS	Cry1Ac+Cry1F	6.0 c	30.12b	3.41abc	14.13d	495.17c	18.83b	61.00c	42.20b	54.87b
F (treatment)		35.78**	60.77**	7.18**	49.71**	110.70**	34. 58**	3.98**	40.40**	24.28**
CV (%)		2.21	4.61	8.26	7.57	5.19	14.41	18.09	3.87	3.69

*Means followed by the same letter do not differ according to Tukey's test at 5% probability. POP: preoviposition period, OP: oviposition period, POPo: postoviposition period, DOC: daily oviposition capacity, TOC: total oviposition capacity, EWP: eggs without pedicels, EV: egg viability, LF: longevity of females and LM: longevity of males. ** Values significant at 0.01% probability according to the ANOVA F test. CV: Coefficient of variation of the data obtained.
Source: research data.

The oviposition period of *C. externa* was influenced by the nutrition of the prey offered to the larvae of this predator, which was reduced compared with that of the FM 993 conventional cultivar. There were significant differences between the technologies used, with Bollgard I having the greatest negative influence on the oviposition period of females that received prey from the DP 90 B and DP 555 BGRR cultivars during the larval stage, with reductions of 36.71% and 41.58%, respectively.

According to an isolated analysis of each technology, only the cultivars containing the Cry1Ac protein differed significantly in the oviposition period of *C. externa* females (Table 1). The females with the longest postoviposition period were those nourished by nymphs reared on the cultivar PHY 440 W (4.00 days), which expresses the combination of Cry1Ac+Cry1F proteins, differing significantly from DP 90 B and DP 604 BG (3.00 days), which are both cultivars expressing the Cry1Ac protein (Table 1).

The daily and total egg-laying capacity of *C. externa* was affected by the type of food provided during the larval stage. The highest number of eggs/female was observed when the larvae consumed aphids from the conventional cultivar FM 993, with 21.08 and 597.50 eggs/day, respectively (Table 1). These two capacities (daily and total) were reduced when lacewing larvae were fed the genetically modified cultivars expressing the Cry1Ac proteins (DP 90 B, ACALA DTL 90 B, DP 555 BGRR), Cry1Ac+Cry2Ab (DP 1228 B2RF), and Cry1Ac+Cry1F (FM 975 WS) (Table 1), indicating that for these two variables (DOC and TOC), the effect of all the toxin combinations had an effect on the reduction in days. The reduction in daily oviposition capacity interfered with total oviposition, ranging from 597.50 to 234.75 chrysopid eggs.

The Bt technologies showed significant differences, with the lowest daily and total oviposition capacity of the females observed when the larvae were fed prey from Bollgard I - Cry1Ac cotton plants (DP 90 B and DP 555 BGRR). The width of the Widestrike - Cry1Ac+Cry1F group did not differ from that of the Bollgard II - Cry1Ac+Cry2Ab2 group but differed significantly between the cultivars (Table 1).

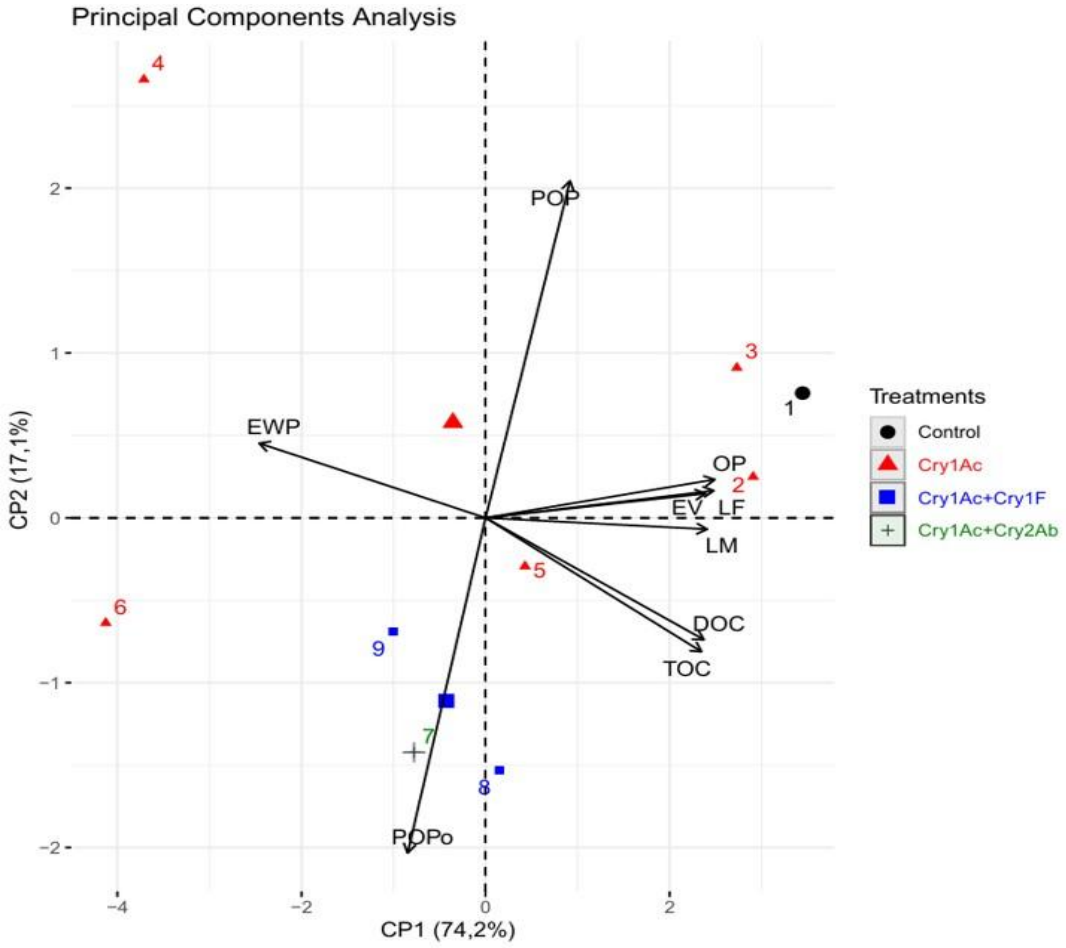
C. externa larvae that fed on aphids from transgenic plants had higher percentages of eggs without pedicels (Table 1). The different Bt proteins tested in isolation revealed that the females

that were fed a diet with aphids from the same technology, Bollgard I® - Cry1Ac, produced higher percentages of eggs without pedicels than the conventional one did (DP 90 B and DP 555 BGRR), ranging from 30.75 to 9.12%, respectively. In terms of egg viability, all the technologies tested (Bollgard I® - Cry1Ac, Bollgard II® - Cry1Ac+Cry2Ab2 and WideStrike® - Cry1Ac+Cry1F) had a negative influence on the hatching of larvae, differing significantly from conventional technologies, except for Bollgard I® on the cultivars DP 604 BG and NuOpal.

With respect to the longevity of *C. externa* (Table 1), adults whose larvae received aphids from Bt cultivars, with the exception of DP 604 BG and NuOpal, had lower longevity. Among the Bt technologies, there were significant differences in the longevity of *C. externa*, where Bollgard I showed differences between the cultivars, where the larvae that consumed prey from the cultivars DP 90 B and DP 555 BGRR provided lower longevities of females and males of *C. externa*. A longer oviposition period and greater oviposition capacity are associated with greater longevity. The longest-lived females had a longer oviposition period and consequently greater daily and total oviposition capacity, whereas the opposite was observed for the shortest-lived females (Table 1). It was also observed that the females with the lowest longevity oviposited the highest percentages of eggs without pedicels and with the lowest viability, which suggests a possible negative interference of these technologies on the reproductive characteristics of *C. externa.*

Principal component (PC) analyses were carried out on the basis of nine variables related to the relationships between *C. externa* fed during immature stages and *A. gossypii* from cotton cultivars with different Bt technologies (Figure 1). A total of 91.3% of the variability of the data obtained was retained in the first two components, and cultivars 2 (DP 604 BG) and 3 (NUOPAL), both expressing the Cry1Ac protein, behaved very similarly to the conventional cultivar (cultivar 1 FM 993) in terms of POP, OP, EV, LF and LM. However, cultivars 4 (DP 90 B) and 6 (DP 555 BGRR), although also expressing the same Cry1Ac protein, presented completely different behavior from the previous cultivars, influencing most of the variables in an inversely proportional way to the behavior of the conventional cultivar, with greater emphasis on the EWP variable (Figure 1).

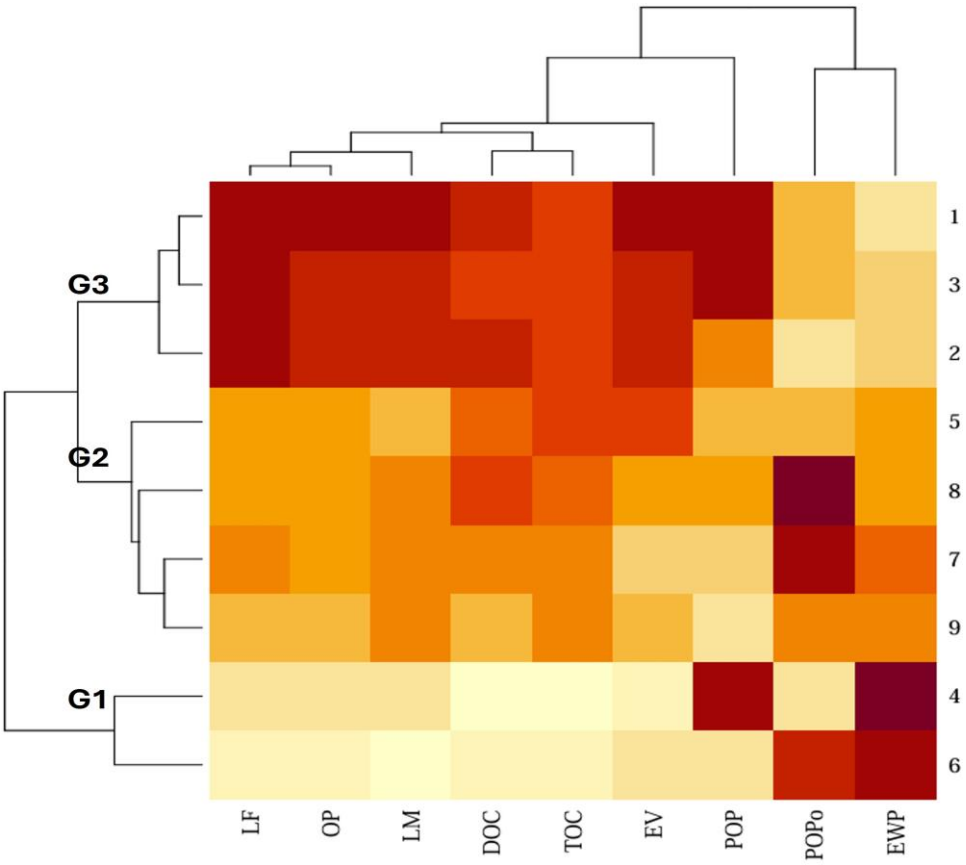
Figure 1 - Principal component analysis (PCA) obtained when evaluating C. *externa* fed during immature stages with *A. gossypii* from cotton cultivars with different Bt technologies. POP: preoviposition period, OP: oviposition period, POPo: postoviposition period, DOC: daily oviposition capacity, TOC: total oviposition capacity, EWP: eggs without pedicels, EV: egg viability, LF: longevity of females and LM: longevity of males. The numbers represent the cultivars used, as described in the Materials and Methods. The different colors represent the different protein combinations present in each genotype



Source: research data.

Cultivars 7 (DP 1228 B2RF with the Cry1Ac+Cry2Ab protein), 8 (PHY 440 W) and 9 (FM 975 WS), the latter two with the Cry1Ac+Cry1F protein, behaved very similarly, being positioned close to the POPo variable, indicating that they influence this variable in the opposite direction to the conventional one. These results confirmed that the different Bt technologies influenced the performance of *C. externa* fed *A. gossypii* during the immature stages.

Figure 2 - Heatmap obtained via the UPGMA clustering method when evaluating *C. externa* fed during immature stages with *A. gossypii* from cotton cultivars with different Bt technologies. POP: preoviposition period, OP: oviposition period, POPo: postoviposition period, DOC: daily oviposition capacity, TOC: total oviposition capacity, EWP: eggs without pedicels, EV: egg viability, LF: longevity of females and LM: longevity of males. The numbers represent the cultivars used, as described in the Materials and Methods. Groups G1 to G3 represent the genotype groupings when considering the variables evaluated



Source: research data.

To complement the results obtained, heatmap analysis was carried out on the basis of the information collected on nine variables obtained when evaluating *C. externa* fed during the immature stages with *A. gossypii* from cotton cultivars with different Bt technologies (Figure 2). The clustering shows that three groups are formed among the cultivars used. A first but divergent group (G1) is formed by cultivars 4 (DP 90 B) and 6 (DP 555 BGRR), both of which express the Cry1Ac protein. These cultivars differ from the other cultivars in that they present lower values for the variables LF, OP, LM, DOC, TOC and EV and higher values for POPo and EWP, confirming

the results in Figure 1. Both cultivars had relatively high numbers of eggs without pedicels, which directly affects the survival of the species in the field due to greater exposure to natural enemies, as well as setting back an evolutionary aspect of the species.

A second group (G2) was formed by cultivars 5 (ACALA DTL 90B - Cry1Ac), 7 (DP 1228 B2RF - Cry1Ac+Cry2Ab), 8 (PHY 440 W - Cry1Ac+Cry1F) and 9 (FM 975 WS - Cry1Ac+Cry1F). This group shows intermediate behavior in relation to the nine variables evaluated, estimated by the intensity of the color shown in Figure 2. The third group (G3) included the conventional cultivar used as control 1 (FM 993) and cultivars 2 (DP 604 BG) and 3 (NUOPAL), both of which expressed the Cry1Ac protein. These cultivars contrasted with the first group, with higher values for the LF, OP, LM, DOC, TOC and EV variables and lower values for POPo and EWP. This grouping was also confirmed by the PC analysis (Figure 1) and shows the complementarity that can be obtained when considering both analyses, thus facilitating the conclusions and interpretation of the results obtained in this work.

In general, few studies in the literature have reported the behavior of arthropods at the third tritophagous level in relation to transgenic plants, especially studies that prove the impact of different Bt cotton technologies in relation to nontarget arthropods such as *A. gossypii* and their influence on the nutrition of the young and adult stages of *C. externa* (Mota *et al.,* 2012; Shera; Karmakar; Sharma*,* 2018). The use of Bt technologies, in which plants express genes encoding different insect toxins from the soil bacterium *Bacillus thuringiensis*, has become increasingly widespread (Waghmare, 2022). In cotton, the first generation of Bt cotton carried a single Bt gene, while the second- and third-generation Bt cotton contained two or three genes for better control of lepidopteran insects (Ahmad *et al.,* 2021). Bt proteins in genetically modified crops can pose a risk to insects, such as nontarget beneficial arthropods, which can act as biological controls and pollination and decomposition services in the ecosystem (Shera; Karmakar; Sharma*,* 2018).

This study revealed that different reproductive stages of *C. externa* that were fed during the immature stages were affected by *A. gossypii* from cotton cultivars with different Bt technologies (Cry1Ac, Cry1Ac+Cry2Ab and Cry1Ac+Cry1F).

The oviposition period was verified via the variables POP, OP, POPo, DOC and TOC, which revealed that among the cultivars used, Cry1Ac technology most affected the performance of *C. externa* associated with the cultivars DP 90 B and DP 555 BGRR (Figures 1 and 2). In this case, it is speculated that the content of aldehyde terpenoid compounds, such as the gossypol content, is

involved, although it was not determined in this study; this chemical factor alone or in combination with others may be involved in the negative effects on the prey and consequently on the predator (Meyer; Vorster; Dubery, 2004; Albuquerque, 2009). These two cultivars differed from the other cultivars evaluated and from the control, with the lowest values for TOC, DOC and OP and higher values for POP and POPo. Santos (2003), evaluating the reproductive potential of *C. externa* according to the diet offered during the larval stage, 3rd- and 4th-instar nymphs of *A. gossypii*, reported a preoviposition period of 4.8 days, which was much shorter than that reported in the present study. The oviposition period presented by these authors was 46 days, and the average total number of eggs was 786.6. These results are higher than those reported in this study.

Consistent with this research, Principi and Canard (1984) and Rousset (1984) reported that the reproductive potential of lacewings can be affected by the quality and quantity of the food ingested in both the adult and larval stages. The provision of poor-quality prey results in smaller imagos with low reproductive viability, with negative effects on the length of oviposition periods, fecundity and fertility.

An indirect effect of Bt technology was detected in this study; however, the effects of the Cry1Ac protein on cultivars differed from those of the conventional control (FM 993), as cultivars expressing the Cry1Ac protein (DP 604 BG and NUOPAL) behaved similarly to the conventional control. This result points to the possibility of the absence of terpenoid aldehyde compounds, such as gossypol, so the presence of the Cry1Ac protein does not always determine the same response, as, for example, observed in the cultivars DP 555 BGRR and DP 90B (Table 1). Morando *et al.* (2014) reported that the Nuopal cultivar presented a relatively high rate of *A. gossypii* infestation, which is likely the case in this study.

The other combinations of Cry1Ac+Cry2Ab and Cry1Ac+Cry1F proteins showed intermediate performance (Figures 1 and 2), although they were different from the control, indicating that the magnitude of the effect of these proteins when combined with Cry1Ac and the respective genetic makeup of the materials did not maximize the effect. Further studies could elucidate the effects of these combinations on the development of *C. externa.*

The nutrition of chrysopid larvae influences the reproductive aspects of the adult because, according to Rousset (1984), previtellogenesis in females can occur before the adult emerges. In the case of males, spermatogenesis occurs in the young stage, so low-quantity and poor-quality prey can lead to the sterilization of the adult, as observed in another chrysopid species, *Chrysopa perla*

(Linnaeus) (Neuroptera: Chrysopidae) (Canard 1970; Albuquerque; Tauber; Tauber, 2012).

Inadequate nutrition for the larval stage cannot be met by adequate nutrition for the adult (Albuquerque, 2009; *Pinto et al., 2019*). Taking this research into account, the feeding habits of *C. externa* differ in the young and adult stages, emphasizing that the negative effects observed (lower oviposition capacity, low egg viability and eggs without pedicels) of some individuals are possibly related to the host plants (Bt cotton) where the aphids were reared.

The host plant can indirectly influence lacewings when the prey stores toxic substances sequestered from the plant species on which it is nourished, acting as a defense against natural enemies and altering its chemical composition (Bowers, 1990; Parrott, 1990; Rowell-Rahier; Pasteels, 1992; Hussain *et al.,* 2019).

In cases where prey have the same chemical composition in relation to nutrients, i.e, they have all the nutritional sources, among other compounds in their metabolism, the concentrations of these nutrients and access to the predator are variable between species (Florkin; Jeuniaux, 1974; Cohen, 1998, Sahayaraj; Hassan, 2023; Fischer *et al.,* 2024).

These intrinsic factors are related to insect physiology, such as adaptability to the diet, reproductive potential, fecundity and fertility, which in turn are influenced by the nutritional requirements of insects (Carvalho; Souza, 2000). New (1975) stated that the quality of feeding during the larval stage can affect the reproductive aspects of subsequent generations. Recent studies have shown that the Bt toxin can persist at the third trophic level in lacewing larvae, affecting their growth indirectly (Guan *et al.,* 2022), which reaffirms the results obtained in this study.

In general, males tended to have a longer lifespan than females did. According to Macedo *et al.* (2010), this fact is possibly related to the fact that females allocate a large part of their nutritional reserves to egg production, causing nutritional depletion, which is subsequently reflected in their longevity.

With respect to the parameter eggs without pedicels, the highest percentages were observed with Cry1Ac technology in the cultivars DP 90B and DP 555 BGRR. This is important because, according to Smith (1922), the pedicel is an adaptive coevolution and defense against cannibalism by the species, as well as protection against attack by other predators, such as ants.

The genetics of the cotton cultivars tested may negatively affect some reproductive aspects of the nontarget organisms, highlighting the importance of tritrophic interaction studies between the young phase of *C. externa* when consuming *A. gossypii* nourished by these cultivars. However, it is

important to mention that there was variation in the results obtained between the same technology, i.e, the presence of the Cry1Ac protein does not always determine the same response, which reinforces that the intrinsic genetics of the cultivars must be a context that emphasizes the importance of continuing studies with biochemical and molecular analyses of the genetic components of the plants and which compounds are involved in the impacts of the various Bt cotton technologies for this predator feeding on the nontarget pest.

4 Conclusion

The reproductive aspects and longevity of *C. externa* were negatively influenced when the larvae were fed *A. gossypii* from the DP 90 B and DP 555 BGRR cultivars, which express the Cry1Ac protein and other intrinsic and unknown genetic characteristics.

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