

UNIVERSIDADE FEDERAL DE PELOTAS
Instituto de Biologia
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Dissertação

Efeitos letais e subletais de SfMNPV (*Spodoptera frugiperda múltiplos nucleopoliedrovírus*) sobre a lagarta do cartucho em soja Bt e não Bt

Marcelo Roberto Zaksessi

Pelotas, 2021

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**Dedico este trabalho aos meus
familiares e a toda comunidade
Científica e Acadêmica do Brasil.**

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Resumo

ZAKSESKI, Marcelo Roberto. **Efeitos letais e subletais de SfMNPV (*Spodoptera frugiperda* múltiplos nucleopoliedrovírus) sobre a lagarta do cartucho em soja Bt e não Bt.** Orientador: Daniel Bernardi. 2021. 44f. Dissertação (Mestrado em Entomologia) - Instituto de Biologia, Universidade Federal de Pelotas, Pelotas, Pelotas, 2021.

O Brasil atualmente é o maior produtor de soja (*Glycine max* (L.) Merrill) do mundo. No entanto, nos últimos anos vem sofrendo com o ataque da lagarta *Spodoptera frugiperda* (J.E. Smith), considerada a principal praga da cultura do milho (*Zea mays* L.). Na cultura da soja esse fato justifica-se principalmente em função da baixa suscetibilidade da praga a proteína Cry1Ac expressa na soja Bt e também a evolução da resistência a diferentes inseticidas sintéticos. A adoção do nucleopolyhedrovirus (SfMNPV - Baculoviridae: Alphabaculovirus) para o manejo da espécie pode ser uma alternativa para a utilização na cultura da soja. Frente a isso, objetivou-se avaliar em condições de laboratório os efeitos letais e subletais de SfMNPV em neonatas de *S. frugiperda* em soja Bt que expressa a proteína Cry1Ac e soja não Bt (isolina). Após a pulverização com diferentes concentrações de SfMNPV em plantas cultivadas em casa de vegetação, foram avaliados os seguintes parâmetros biológicos: sobrevivência aos dez dias de idade, duração do período de larva até pupa, peso larval e pupal, fecundidade e fertilidade. Ademais, foi calculado a tabela de vida de fertilidade. Os resultados indicaram que neonatas de *S. frugiperda* não sobreviveram após a infecção em soja Bt e não Bt pulverizada com a dose recomendada de campo de SfMNPV (7.50×10^9 corpos de oclusão/mL (OBs) de SfMNPV). Em contraste, neonatas de *S. frugiperda* infectadas com as concentrações mais baixas de SfMNPV [($7,50 \times 10^8$ OBs/mL - 10% da dose de campo, $1,88 \times 10^9$ OBs /mL - 25% da dose de campo e $3,75 \times 10^9$ OBs/mL - 50% da dose de campo)] em soja Bt e não Bt conseguiram completar o ciclo biológico até a fase adulta. Entretanto, lagartas sobreviventes em soja Bt e não Bt quando infectadas com as concentrações de 25% e 50% da dose de campo apresentaram maior tempo de desenvolvimento (larva até pupa), menor peso larval e pupal, além de reduções na fecundidade e fertilidade. Os efeitos subletais na fase larval afetaram negativamente os parâmetros da tabela de vida de fertilidade com uma menor taxa de reprodutiva e um menor crescimento populacional da espécie em soja Bt e não Bt. Os resultados encontrados no presente trabalho revelaram que, além da mortalidade, o bioinseticida contendo SfMNPV também causa toxicidades subletais significativas em neonatas de *S. frugiperda*, o que demonstra um efeito sinérgico entre SfMNPV e soja Bt que expressa a proteína Cry1Ac. Quando utilizado de concentrações recomendadas pelo fabricante, concluiu-se que o uso do vírus SfMNPV se mostrou uma alternativa promissora no manejo de neonatas de *S. frugiperda*, sendo oportuna a sua utilização em programas de manejo integrado de pragas.

Palavras-chave: baculovírus; controle biológico; MIP; Proteína Cry1AC; tabela de fertilidade de vida.

Abstract

ZAKSESKI, Marcelo Roberto. **Lethal and sublethal effects of SfMNPV (*Spodoptera frugiperda* multiple nucleopolyhedrovirus) on fall armyworm in Bt and non Bt soybeans.** Advisor: Daniel Bernardi. 2021. 44 f. Dissertation (Masters in Entomology) – Institute of Biology, Federal University of Pelotas, Pelotas, 2021.

Brazil is currently the largest producer of soy (*Glycine max* (L.) Merrill) in the world. However, in recent years it has been suffering from the attack of the caterpillar *Spodoptera frugiperda* (J.E. Smith), considered the main pest of the maize crop (*Zea mays* L.). In soybean culture, this fact is mainly justified by the low susceptibility of the pest to Cry1Ac protein expressed in Bt soybean and also the evolution of resistance to different synthetic insecticides. The adoption of nucleopolyhedrovirus (SfMNPV - Baculoviridae: Alphabaculovirus) for the management of the species may be an alternative for use in soybean culture. Therefore, this study aimed to evaluate, under laboratory conditions, the lethal and sublethal effects of SfMNPV on *S. frugiperda* neonates on Bt soy expressing protein Cry1Ac and non Bt soy (isoline). After spraying with different concentrations of SfMNPV on plants grown in a greenhouse, the following biological parameters were evaluated: survival at ten days of age, duration of the period from larva to pupae, larval and pupal weight, fecundity and fertility. Furthermore, the fertility life table was calculated. Results indicated that *S. frugiperda* neonates did not survive infection on Bt and non Bt soybeans sprayed with the recommended field dose of SfMNPV (7.50×10^9 occlusion bodies/mL (OBs) of SfMNPV). In contrast, *S. frugiperda* neonates infected with the lowest concentrations of SfMNPV [(7.50×10^8 OBs/mL - 10% of field dose, 1.88×10^9 OBs /mL - 25% of field dose and 3.75×10^9 OBs/mL - 50% of field dose)] in Bt and non Bt soybeans were able to complete the biological cycle to adulthood. However, caterpillars surviving on Bt and non Bt soybeans, when infected with concentrations of 25% and 50% of the field dose, showed longer developmental time (larva to pupae), lower larval and pupal weight, in addition to reductions in fecundity and fertility. Sublethal effects in the larval stage negatively affected the fertility life table parameters with a lower reproductive rate and lower population growth of the species in Bt and non Bt soybeans. The results found in the present work revealed that, in addition to mortality, the bioinsecticide containing SfMNPV also causes significant sublethal toxicities in *S. frugiperda* neonates, which demonstrates a synergistic effect between SfMNPV and Bt soy expressing the Cry1Ac protein. When used at concentrations recommended by the manufacturer, it was concluded that the use of the SfMNPV virus proved to be a promising alternative in the management of *S. frugiperda* neonates, and its use in integrated pest management programs is opportune.

Key words: baculovirus; biological control; MIP; Cry1AC protein; life fertility table.

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1. Introdução geral

Atualmente o Brasil é considerado o maior produtor de soja (*Glycine max* (L.) Merrill) (Fabaceae: Phaseoleae) do mundo, com uma área estimada de cultivo de aproximadamente 37 milhões de hectares (safra 2019/2020) (CONAB, 2020). Os avanços biotecnológicos têm proporcionado grandes benefícios a cultura nos últimos anos, principalmente, em função do melhoramento genético, o qual aumentou o potencial produtivo da cultura. Entretanto, um dos principais problemas associados à produção sojícola, além de fatores abióticos como disponibilidade hídrica, temperatura e radiação solar, são os fatores bióticos, como ocorrência de doenças, nematoides, competição com plantas daninhas, ácaros e artrópodes-praga, que aumentam os custos de produção relacionados ao manejo, comprometem a qualidade dos grãos e principalmente a produtividade (HOFFMANN-CAMPO *et al.*, 2012).

Em relação aos artrópodes-praga, no Brasil, destacam-se algumas espécies da ordem Lepidoptera, como: *Helicoverpa armigera* Hübner, 1809, *Chrysodeixis includens* Walker, 1858, (Lepidoptera: Noctuidae), *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Erebidae) e *Elasmopalpus lignosellus* Zeller, 1848 (Lepidoptera: Pyralidae). No entanto, tem sido observado um aumento na ocorrência de espécies do gênero *Spodoptera* (BUENO *et al.*, 2011; JUSTINIANO; FERNANDES; VIANA, 2014; BLANCO *et al.*, 2016; CONTE *et al.*, 2019). Na cultura da soja, dentre as espécies deste gênero destacam-se a *Spodoptera frugiperda* (J.E. Smith), *Spodoptera eridania* (Cramer), *Spodoptera cosmioides* (Walker) e *Spodoptera albula* (Walker) (Lepidoptera: Noctuidae) (HOFFMANN-CAMPO *et al.*, 2012; SILVA *et al.*, 2017).

Conhecida como lagarta-do-cartucho do milho ou lagarta militar, a ocorrência em soja vem aumentando, principalmente, em função da diversidade de hospedeiros e elevada polifagia da espécie (PERUCA, 2015), uma vez que, historicamente, a presença era mais comum em plantas da família Poaceae, como o milho (*Zea mays* L.), milheto (*Pennisetum glaucum* (L.)), trigo (*Triticum* spp.), sorgo (*Sorghum* spp.), arroz (*Oryza sativa* L.) e cana-de-açúcar (*Saccharum* spp. L.) (CRUZ, 1995). No Brasil, praticamente em todas as regiões produtoras de soja é possível de encontrar essa espécie nos campos de produção (BUENO *et al.*, 2011; JUSTINIANO; FERNANDES; VIANA, 2014; BLANCO *et al.*, 2016; CONTE *et al.*, 2019). Esse fato justifica-se, principalmente, pela habilidade de adaptação a diferentes latitudes e longitudes (CRUZ, 1995). Na soja, *S. frugiperda* causa danos desde o período

vegetativo até o reprodutivo, reduzindo o estande inicial de plantas, desfolha, e também causando danos nas inflorescências e legumes (BARROS; TORRES; BUENO, 2010; HOFFMANN-CAMPO *et al.*, 2012; SILVA *et al.*, 2017).

Na produção de grãos em larga escala, a principal estratégia de controle de espécies de *Spodoptera* é por meio do uso de inseticidas químicos (BERNARDI *et al.*, 2014b; LUZ *et al.*, 2018). Entretanto, o uso intensivo de inseticidas para o controle de *S. frugiperda* tem ocasionado um aumento no número de casos de resistência a diversos grupos de inseticidas, como para lambda-cialotrina (DIEZ-RODRÍGUEZ; OMOTO, 2001), clorpirifós (CARVALHO *et al.*, 2013), lufenurom (NASCIMENTO *et al.*, 2016), espinosade (OKUMA *et al.*, 2018) e clorantraniliprole (BOLZAN *et al.*, 2019). Fato que ocorreu também com o uso de plantas Bt que expressam diferentes proteínas de *Bacillus thuringiensis*, como em milho para a proteína Cry1F (FARIAS *et al.*, 2014; SANTOS-AMAYA *et al.*, 2015), proteína Cry1Ab (OMOTO *et al.*, 2016), Cry1A.105/Cry2Ab2 (BERNARDI *et al.*, 2015; SANTOS-AMAYA *et al.*, 2015), Cry1A.105/Cry2Ab2/Cry1F (BERNARDI *et al.*, 2016a; HORIKOSHI *et al.*, 2016), e Vip3Aa20 e Vip3Aa20/Cry1Ab, (BERNARDI *et al.*, 2016b).

Na cultura da soja, a partir de 2010 houve a liberação do primeiro evento de soja Bt, expressando Cry1Ac (evento MON 87701 × MON 89788). Estudos demonstraram que essa proteína apresenta elevada toxicidade sobre lepidópteros-praga considerados primários para a cultura, como *A. gemmatalis* e *C. includens* (BERNARDI *et al.*, 2012), *Chloridea virescens* (BERNARDI *et al.*, 2014a) e *H. armigera* (DOURADO *et al.*, 2016). Entretanto, demonstrou baixa toxicidade para espécies do gênero *Spodoptera*, principalmente *S. frugiperda* (BERNARDI *et al.*, 2014b; BORTOLOTTI *et al.*, 2015 SILVA *et al.*, 2017). A baixa toxicidade sobre *S. frugiperda* tem-se agravado também devido a ocorrência de resistência cruzada com as proteínas Cry1Ab e Cry1F expressas no milho e algodão Bt (BERNARDI *et al.*, 2015; HORIKOSHI *et al.*, 2016; OMOTO *et al.*, 2016; SANTOS-AMAYA *et al.*, 2017).

Frente a este cenário, a utilização de produtos biológicos a base de vírus entomopatogênicos podem ser uma alternativa sustentável para incorporar dentro dos programas de Manejo Integrado de Pragas (MIP) (MOSCARDI, 1999; BUENO *et al.*, 2012). Este fato pode ser creditado aos avanços na produção e formulação de produtos à base de baculovírus no manejo de pragas (MOSCARDI, 1999; BEAS-CATENA *et al.*, 2014). No Brasil, durante a década de 1980, o uso do baculovírus *Anticarsia gemmatalis* NPV múltiplo (AgMNPV) em soja foi reconhecida mundialmente

por seu sucesso no manejo biológico do inseto (MOSCARDI, 1999). Entretanto, o uso de AgMNPV diminuiu ao longo do tempo devido a introdução de inseticidas químicos altamente eficazes para seu controle (BEAS-CATENA *et al.*, 2014). O uso de baculovírus nos programas de MIP destaca-se como uma ferramenta de manejo devido à sua elevada eficácia, especificidade sobre o alvo de controle, seletividade a insetos não-alvo e segurança para os seres humanos e/o meio ambiente (MOSCARDI, 1999, BEAS-CATENA *et al.*, 2014).

No Brasil, em 2017 houve o registro dos vírus entomopatogênicos pelo Comitê de Ação de Resistência a Inseticidas (IRAC), com um novo modo de ação (Grupo 31). O produto comercial Cartugem™ (SfMNPV) registrado para o uso no manejo de *S. frugiperda* pode ser uma alternativa para o manejo de *S. frugiperda* na cultura da soja. Ademais, além da toxicidade letal sobre o alvo, estudos têm demonstrado que os inseticidas a base de vírus podem ocasionar efeitos subletais nas espécies contaminadas, como, desenvolvimento prolongado e redução das fases larval e pupal, alteração na razão sexual e redução da fecundidade dos adultos após as lagartas ingerirem o vírus (MONOBRULLAH; SHANKAR, 2008; WILLIAMS *et al.* 2017). Dentro do MIP, essas interferências negativas no desenvolvimento do ciclo biológico das espécies após a ingestão do vírus é benéfico para que ocorra uma supressão da densidade populacional da praga ao longo do tempo (FÜHR *et al.*, 2020).

Embora, o vírus SfMNPV se caracterize devido a com alta especificidade e potencial para uso em no manejo de *S. frugiperda* (VALICENTE; TUELHER, 2009; BARRERA *et al.*, 2011) e elevada toxicidade em lagartas da espécie em laboratório (BENTIVENHA *et al.*, 2019), sua introdução para uso em larga escala comercial devem ser avaliadas para delinear o posicionamento correto desta alternativa de manejo. De posse do exposto, o objetivo do trabalho foi avaliar a toxicidade letal e subletal do vírus SfMNPV sobre *S. frugiperda* quando aplicado em diferentes dosagens em soja Bt e não Bt.

Artigo 1 - A ser submetido na revista Journal of Economic Entomology
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Toxicity assessment of SfMNPV-based biopesticide on *Spodoptera frugiperda* (Lepidoptera: Noctuidae) developing on transgenic soybean expressing Cry1Ac insecticidal protein

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Abstract - Toxicity assessment of a baculovirus-based biopesticide containing *Spodoptera frugiperda* multiple nucleopolyhedrovirus (SfMNPV: *Baculoviridae*: *Alphabaculovirus*) infecting fall armyworm, *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae) is reported. In the bioassays, neonates were infected with different doses of SfMNPV applied on Cry1Ac Bt soybean and non-Bt soybean. Our findings indicated that *S. frugiperda* neonates did not survive at 10 d post infection or develop into adults on Bt and non-Bt soybean sprayed with the field recommended dose of SfMNPV. In contrast, a proportion of the infected neonates developed into adults when infected with lower doses of SfMNPV (50%, 25% and 10% of field dose) in both Bt and non-Bt soybean. However, *S. frugiperda* neonates surviving infection at the lowest virus doses on both soybean varieties showed longer neonate-to-pupa and neonate-to-adult periods, lower larval and pupal weights, reduced fecundity, and increased population suppression. Nevertheless, more pronounced toxicities of SfMNPV infecting neonates of *S. frugiperda* were verified on larvae that developed on Bt soybean. These findings revealed that, beyond mortality, the biopesticide containing SfMNPV also causes significant sublethal toxicities on neonates of *S. frugiperda* developing on Bt and non-Bt soybean and suggested a synergic effect among SfMNPV and Cry1Ac insecticidal protein expressed in Bt soybean.

Keywords: fall armyworm, Bt crops, baculovirus, sublethal toxicity, life history traits

Introduction

The deployment of transgenic crops containing insecticidal proteins from *Bacillus thuringiensis* (Bt) Berliner providing resistance against key pest species of several crops revolutionized pest management worldwide. Brazil is one of the top five countries with high

adoption of biotech crops, cultivating more than 51 million hectares in 2019 (James 2019). Brazil was the first country to cultivate Bt soybean [*Glycine max* L. (Merr.)] to control pest species worldwide (CTNBio 2010). Currently, three *Bt*-soybean technologies are available commercially in Brazil; an event expressing a single Cry1Ac protein (Bernardi et al. 2012) and two pyramided events expressing Cry1Ac/Cry1F (Marques et al. 2017) and Cry1Ac/Cry1F/Cry2Ab2 (Bacalhau et al. 2020) proteins. During the 2019 soybean season, nearly 25 million hectares were planted with Bt soybeans (still only Cry1Ac-soybean), representing more than 65% of the total soybean area in Brazil (Brookes and Barfoot 2020).

The current Bt soybean technologies provide effective control of the most important lepidopteran pests that attack soybean. The effectiveness of Bt soybeans has been reported for key lepidopteran pests, such as *Anticarsia gemmatalis* (Hübner, 1818) (Lepidoptera: Erebidae), *Chrysodeixis includens* (Walker, 1858), *Helicoverpa armigera* (Hübner, 1805), and *Chloridea virescens* (F. 1777) (Lepidoptera: Noctuidae) (Bernardi et al. 2012, Yano et al. 2016, Dourado et al. 2016, Marques et al. 2017). However, current Bt soybean technologies have minimal activity against *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae) – an important emerging lepidopteran pest on Bt and non-Bt soybean in Brazil (Bernardi et al. 2014, Marques et al. 2017, Machado et al. 2020). The increase of *S. frugiperda* on soybean can be attributed to its low natural susceptibility to Bt proteins expressed on soybean, field-evolved resistance to some Bt proteins, and adaptation to the Brazilian cropping system (Bernardi et al. 2014, Farias et al. 2014, Omoto et al. 2016, Machado et al. 2020).

Spodoptera frugiperda in advanced instars cuts plants right after emergence and causes defoliation and damage to flowers and pods of soybean, requiring the adoption of chemical or biological control to protect yields (Bueno et al. 2011, Moscardi et al. 2013). Since 2018, a baculovirus-based biopesticide containing *Spodoptera frugiperda multiple*

nucleopolyhedrovirus (SfMNPV; *Baculoviridae: Alphabaculovirus*) has been used in Brazil as an alternative to synthetic insecticides against *S. frugiperda* that infest Bt and non-Bt crops. Previous studies have demonstrated that the baculovirus-based insecticide containing SfMNPV was effective against *S. frugiperda* (Barrera et al. 2011) and presented no cross-resistance with synthetic insecticides or Bt-plants expressing insecticidal proteins (Bentivenha et al. 2019).

Given the promising adoption of the SfMNPV-based biopesticide for controlling *S. frugiperda* in Bt and non-Bt crops in Brazil, it is essential to understand the insect-pathogen interactions. Based on this, the objective of the present study was to evaluate the toxicity of the SfMNPV-based biopesticide on *S. frugiperda* surviving on Bt soybean expressing Cry1Ac protein and non-Bt soybean. Our hypothesis is that the toxicity of the baculovirus-based biopesticide infecting *S. frugiperda* is more pronounced on larvae developing on Cry1Ac-soybean.

Material and Methods

Insect Source. A population of *S. frugiperda* collected in non-Bt maize during the 2012 crop season in Mogi Mirim, São Paulo, Brazil (22°28'31" S and 46°54'21" W) was used as the source of insects. This population is considered a susceptible reference for studies with insecticides and Bt proteins. In laboratory conditions, larvae were maintained on artificial diet adapted from Greene et al. (1976).

Plants. Seeds of Cry1Ac-soybean (BMX Ponta IPRO, Brasmax, Passo Fundo, RS, Brazil) and a non-Bt soybean (BMX Valente 6968 RSF, Brasmax, Passo Fundo, RS, Brazil) of the maturity group 6.6 were sown in 12-L plastic pots (3 seeds/pot) containing soil substrate. Pots were

maintained in a greenhouse. At sowing, Nitrogen–Phosphorus–Potassium (NPK; 5–25–25) was applied according to the technical recommendations for soybean.

Bioassays. To perform bioassays, the commercial product Cartugen[®] (active ingredient SfMNPV, concentration 7.50×10^9 occlusion bodies (OBs)/mL) was provided by AgBiTech, TX, USA. At the V₄ phenological stage, Bt and non-Bt soybean plants were sprayed with the commercial-based product containing SfMNPV diluted in water at the following doses: 0 (control – unsprayed leaves), field recommended dose (100 mL Cartugen[®]/100L water – equivalent to 1.50×10^{10} occlusion bodies (OBs)/L), 50% (7.50×10^9 OBs/L), 25% (3.75×10^9 OBs/L), and 10% (1.50×10^9 OBs/L) of the field dose. Application of the different doses of SfMNPV was carried out using a 5-L capacity sprayer equipped with a cone-type nozzle (XR 110.02 fan-type nozzle tips). After two hours, leaves from the upper third of the soybean plants were removed and transported to the laboratory. Leaves were cut into pieces ($\sim 5 \text{ cm}^2$) and conditioned over a gelled layer of 2.5% agar-water in 16-well plastic plates (Advento do Brasil, São Paulo, Brazil). Each well was infested with a single neonate of *S. frugiperda* in a total of 10 replicates (plates) of 16 neonates per treatment ($n=160$). Plates were closed and maintained in a chamber at $27 \pm 2^\circ\text{C}$, $65 \pm 10\%$ RH, and 14:10 h photoperiod. Larvae were fed on soybean leaves sprayed with SfMNPV during 5 d. Then, unsprayed leaves were provided every 48 h until pupation. The following biological parameters of immature stages were evaluated: survival at 10 d and at eclosion, duration of neonate-to-pupation and neonate-to-eclosion periods, larval weight at 12 d post-infection (d.p.i.), and pupal weight 24 h after pupal formation. To measure fecundity, eight to 15 couples/treatment were paired separately and used to quantify the number of eggs per female. Each pair was maintained in PVC cages (23-cm height \times 15-cm diameter) internally coated with a white paper and closed with a sheer fabric.

Statistical Analyses. To assess the lethal and sublethal effects of SfMNPV on *S. frugiperda* developing on Bt and non-Bt soybean, data from all biological parameters evaluated were subjected to a two-way ANOVA using the PROC GLM procedure in SAS[®] 9.1 (SAS Institute 2000). The factor A was represented by one Bt soybean plant (expressing Cry1Ac) and one non-Bt soybean. The factor B was composed by four doses of SfMNPV-based product (100%, 50%, 25%, and 10% of field recommended rate) and untreated controls. Soybeans, doses of SfMNPV, and interactions were used as fixed factors in the model. Mean differences were estimated by Least-Square Means Statement (LSMEANS option of PROC GLM) using a Tukey-Kramer adjustment test ($P < 0.05$) in SAS[®] 9.1 (SAS Institute 2000). Survivorship, development time, and reproductive data were used to estimate population growth parameters, such as mean length of a generation (T), net reproductive rate (R_0 ; average number of female offspring that would be born to a cohort of females), and intrinsic rate of population increase (r_m ; daily production of females per parental female). Fertility life table parameters were obtained using the jackknife technique applying the “*lifetable.sas*” procedure developed by Maia et al. (2000) in SAS[®] 9.1 (SAS Institute 2002).

Results

Survivorship. The main effects of SfMNPV dose, soybean, and soybean \times SfMNPV dose were all significant for neonate survival at 10 d.p.i. ($F = 462.00$; $df = 4, 90$; $P < 0.0001$; $F = 617.51$; $df = 1, 90$; $P < 0.0001$; $F = 117.82$; $df = 4, 90$; $P < 0.0001$, respectively). There were no survivors beyond 10 d.p.i on Bt and non-Bt soybean leaves sprayed with the field dose of SfMNPV (Table 1). The larval survivorship at 10 d.p.i. on Bt soybean at the lower SfMNPV doses (50%, 25% and 10% field dose) differed from each other, with survival rates ranging from 10.48% to 36.55%. For the matching time evaluation and dose rates, survivorship on

non-Bt soybean also varied significantly, ranging from 17.57% to 74.21%. In contrast, a higher survivorship was verified on unsprayed leaves of Bt and non-Bt soybean (66.18% and 91.61%, respectively).

A significant interaction between the soybean and SfMNPV dose ($F = 115.59$; $df = 4, 90$; $P < 0.0001$) was verified for survivorship at eclosion. The main effects of soybean ($F = 578.88$; $df = 1, 90$; $P < 0.0001$) and dose of SfMNPV ($F = 413.49$; $df = 4, 90$; $P < 0.0001$) for this variable were also significant. Similar to larval results, no individuals survived to eclosion on Bt and non-Bt soybean treated at the highest virus dose (Table 1). The survivorship at eclosion varied significantly between the lower doses of SfMNPV (50%, 25% and 10% field dose), ranging from 9.92% to 34.36% on Bt soybean and from 15.99% to 71.04% on non-Bt soybean, whereas the survivorship on controls (unsprayed leaves) was 64.38% and 89.62%, respectively. When larvae were fed soybean varieties with the same SfMNPV dose, survival at 10 d.p.i. and at eclosion was consistently lower in larvae fed Bt soybeans (Fig. 1A).

Development. There were statistically significant effects of the SfMNPV dose ($F = 132.88$; $df = 3, 72$; $P < 0.0001$), soybean ($F = 109.51$; $df = 1, 72$; $P < 0.0001$), and soybean \times dose of SfMNPV ($F = 8.50$; $df = 3, 72$; $P < 0.0001$) on the duration of the neonate-to-pupation period. This period was ~14 and 7 d longer for neonates developing on Bt soybean sprayed with 50% and 25% of the field SfMNPV dose, respectively, than on the lowest virus dose and control treatment (unsprayed) (Table 2). In contrast, on non-Bt soybean this same period was only 7–10 d longer for neonates developing on leaves sprayed with 50% of the field dose (Table 2). The neonate-to-pupation period for larvae exposed at the same virus dose on Bt soybean and non-Bt soybean was consistently longer for those developing on Bt soybean (Fig. 1B).

The effect of SfMNPV dose, soybean, and soybean \times dose on the duration of the neonate-to-eclosion period was significant ($F = 146.06$; $df = 3, 72$; $P < 0.0001$, $F = 137.25$; $df = 1, 72$;

$P < 0.0001$, $F = 9.60$; $df = 3, 72$; $P < 0.0001$, respectively). The neonate-to-eclosion period was from 16 to 8 d longer for *S. frugiperda* developing on Bt soybean sprayed with 50% and 25% of the field dose rate, respectively, compared to the lowest dose and control (unsprayed leaves) (Table 3). Similar results were observed for neonates developing on non-Bt soybean sprayed with 50% and 25% of the field dose rate of SfMNPV, which presented from ~12 and 7 d longer neonate-to-eclosion period when compared to 10% of the field virus dose and control treatment (Table 2). For this variable, there was also a longer development time for neonates feeding on Bt soybean in all SfMNPV doses (Fig. 1B).

There was not a soybean \times virus dose interaction for the larval weight measured at 12 d.p.i. ($F = 0.81$; $df = 3, 72$; $P < 0.0001$). In contrast, the main effects of soybean ($F = 181.59$; $df = 1, 72$; $P < 0.0001$) and dose ($F = 21.79$; $df = 3, 72$; $P < 0.0001$) were significant. Larvae that developed on Bt soybean and were infected by 50% and 25% of the virus field dose (38.8 and 42.1 mg/larva, respectively) had lower weights than neonates exposed to the lowest dose or control (50.7 and 69.2 mg/larva, respectively) (Table 2). Comparing the same SfMNPV doses applied on both Bt and non-Bt soybean it was verified that Bt soybean fed larvae consistently had lower weights (Fig. 1C).

Pupal weights were also significantly affected by the soybean \times dose of SfMNPV interaction ($F = 25.21$; $df = 3, 72$; $P < 0.0001$), soybean ($F = 71.10$; $df = 1, 72$; $P < 0.0001$), and dose of SfMNPV ($F = 134.93$; $df = 3, 72$; $P < 0.0001$). Pupal weights were also lower for larvae fed Bt soybean sprayed with 50% and 25% of the field SfMNPV dose (72.9 and 73.8 mg/pupa) than larvae fed the lowest dose and control treatment (107.5 and 127.4 mg/pupa, respectively) (Table 2). Except for 50% of the SfMNPV field dose, the surviving larvae on Bt soybean produced pupae with lower weights than those that developed on non-Bt soybean (Fig. 1C).

Reproduction and Population Growth. There was a significant soybean \times SfMNPV dose ($F = 12.84$; $df = 3, 71$; $P < 0.0001$), soybean ($F = 77.15$; $df = 1, 71$; $P < 0.0001$), and SfMNPV dose ($F = 30.59$; $df = 3, 71$; $P < 0.0001$) effect in the number of eggs laid per female (Table 2). Survivors from Bt and non-Bt soybean sprayed with 50% and 25% of the virus field rate produced females that laid fewer eggs than females from Bt and non-Bt soybean sprayed with the lowest dose as well as unsprayed leaves (Table 2). Females resulting from surviving larvae exposed to the lowest dose of SfMNPV and unsprayed controls of both soybean plants oviposited a similar number of eggs. However, females from larvae that developed on Bt soybean had a significantly lower fecundity (from 47% to 73% lower eggs laid) than those on non-Bt soybean (Fig. 1D).

According to the estimated life table parameters (Table 3), female progeny resulting from neonates exposed to 50% and 25% of the virus field rate fed Bt soybeans produced fewer than 22 females/newborn female/generation (R_o) in 53 to 59 d (T), whereas progeny survivors at the lowest doses (10% field rate) produced 60 females/female in 45 d, which did not differ from the control (Table 3). When fed non-Bt soybeans, female progeny generated from larvae infected at 50% and 25% of field rate also generated fewer females (18 and 84 females/female, respectively) with a mean generation time >44 d. Surviving neonates that developed on non-Bt soybean infected with 10% of the virus field rate and without infection (control) produced a similar number of females (545 vs 587 females/female) with a generation time of 41 d (Table 3). Female progeny of the 50% and 25% field rate survivors also presented a rate of natural population increase (r_m) lower than 0.057 and 0.099 on Bt and non-Bt soybean, respectively, indicating approximately 50% lower capacity for a population increase than at the lowest doses (Table 3). Neonates that developed on both soybean types and infected with the virus field dose did not produce viable offspring. In all comparisons between Bt and non-Bt soybeans, the population growth parameters were always lower on Bt

soybean than on non-Bt soybean, independently of the SfMNPV dose (Table S1). These findings indicate more pronounced lethal and sublethal effects of SfMNPV applied on Bt soybean expressing Cry1Ac than on non-Bt soybean, causing a greater population suppression of *S. frugiperda*.

Discussion

Lethal and sublethal toxicities of the formulated insecticide containing SfMNPV increased with increasing baculovirus doses when applied on Bt soybean expressing Cry1Ac and non-Bt soybean. Our findings indicate that neonates of *S. frugiperda* exposed to the field recommended dose of SfMNPV did not survive beyond 10 d.p.i. or develop into adults, regardless of the soybean variety. Our results also demonstrated that neonates exposed at the SfMNPV lowest doses applied on Bt soybean expressed more pronounced lethal and sublethal toxicities against *S. frugiperda* than on non-Bt soybean sprayed with 50% and 25% of the field dose, suggesting a synergic effect among SfMNPV and Cry1Ac protein expressed on soybean. The sublethal effects are related with prolonged developmental time of neonate-to-pupation and neonate-to-eclosion periods, reduced larval and pupal weights, decreased fecundity, and increased population suppression. At the lowest dose, neonates of *S. frugiperda* were unaffected by sublethal viral effects in most life history traits evaluated.

Lethal and sublethal toxicities of baculovirus-based insecticides containing nucleopolyhedrovirus (NPVs) have previously been reported in other *Spodoptera* species. Reductions in fecundity was verified in SpltMNPV (*Spodoptera litura* MNPV) infections in *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) (Monobrullah and Shankar 2008), lower fecundity, altered sex ratio and longer larval and pupal phases in *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) infected by *S. exigua* MNPV (SeMNPV) (Cabodevilla et al. 2011),

and alterations in the proportion of males and females in *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) after infection by *Autographa californica* MNPV (AcMNPV) (Scheepens and Wysoki 1989). Sublethal infections by NPVs of other lepidopteran pests was also related with lower fecundity and longer larval and pupal periods in *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) and *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), alterations in sex ratio in *Mamestra brassicae* (L.) (Lepidoptera: Noctuidae), low larval weight, reduced fecundity and fertility, altered sex ratio, and fewer females produced per female in *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae), and reduced pupal weight in *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae) and *Lymantria dispar* (L.) (Lepidoptera: Lymantridae) (Sait el al. 1994, Goulson and Cory 1995, Milks et al. 1998, Murray et al. 1991, Milks et al. 1998, Duan and Otvos 2001, Führ et al. 2021).

The combination of current available Bt soybeans and baculovirus-based products can be considered an ecologically safe and selective tactic against *S. frugiperda* that survive on Bt soybean. According to Bentivenha et al. (2019), Brazilian populations of *S. frugiperda* had similar susceptibility to SfMNPV and also no cross-resistance to chemical insecticides and Bt proteins, appearing as an innovative mode of action for IPM and IRM programs. The high adoption of Cry1Ac-soybean in Brazil (more than 25 million ha per season) with low effectiveness against *S. frugiperda* contribute to the adoption of biopesticides containing baculovirus to complement the control of this pest. Therefore, the integration of IPM tactics with distinct mortality agents, rather than use as single control tactics as Bt plants and chemical insecticides, contribute to ensure the sustainability of the current agricultural system in Brazil, where *S. frugiperda* developed resistance to various Bt toxins (Farias et al. 2014, Omoto et al. 2016) and chemical insecticides (Diez-Rodríguez and Omoto, 2001, Carvalho et al. 2013, Nascimento et al. 2016, Okuma et al. 2018, Bolzan et al. 2019).

In summary, the present study documents that the SfMNPV-based biopesticide Cartugen[®] had lethal and sublethal toxicity against *S. frugiperda*. Our finding indicates that neonates of *S. frugiperda* infected with sublethal quantities of SfMNPV present longer development of immature stages, lower reproductive rate, and suppression of population growth. Considering the promising adoption of SfMNPV against *S. frugiperda* on Bt soybean and other Bt and non-Bt crops, it is important to monitor the occurrence of *S. frugiperda* to define the best time of virus application, which is needed to control first instar larvae. The favorable toxicological profile of baculovirus-based pesticides on beneficial natural enemies also provides opportunities for establishment of IPM and IRM programs.

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Table 1. Survivorship ($\% \pm \text{SE}$) of *S. frugiperda* neonates developing on Bt soybean expressing Cry1Ac protein and non-Bt soybean sprayed with different doses of *S. frugiperda* multiple nucleopolyhedrovirus (SfMNPV).

Dose of SfMNPV ^a	Survivorship at 10 d.p.i. (%) ^b	Survivorship at eclosion (%) ^b
Bt soybean		
0 (control)	66.18 \pm 2.79 a	64.38 \pm 1.62 a
10% of field dose (7.50×10^8 OBs/L)	36.55 \pm 1.37 b	34.67 \pm 1.83 b
25% of field dose (1.88×10^9 OBs/L)	20.45 \pm 1.45 c	19.33 \pm 1.52 c
50% of field dose (3.75×10^9 OBs/L)	10.48 \pm 0.83 d	9.92 \pm 0.87 d
Field dose (7.50×10^9 OBs/L)	0.00 \pm 0.00 e	0.00 \pm 0.00 e
Non-Bt soybean		
0 (control)	91.61 \pm 2.53 a	89.62 \pm 2.85 a
10% of field dose (7.50×10^8 OBs/L)	74.21 \pm 2.39 b	71.04 \pm 2.26 b
25% of field dose (1.88×10^9 OBs/L)	43.51 \pm 2.24 c	42.36 \pm 2.21 c
50% of field dose (3.75×10^9 OBs/L)	17.57 \pm 1.86 d	15.99 \pm 1.52 d
Field dose (7.50×10^9 OBs/L)	0.00 \pm 0.00 e	0.00 \pm 0.00 e

^aThe field dose of SfMNPV used against *S. frugiperda* neonates was 100 mL Cartugen[®]/100L water (representing 7.5×10^9 Occlusion Bodies (OBs)/L water).

^bMeans \pm SE with the same letter in each column and soybean are not significantly different (LSMEANS followed by Tukey test; $P > 0.05$).

Table 2. Biological parameters (mean \pm SE) of *S. frugiperda* developing on Bt soybean expressing Cry1Ac protein and non-Bt soybean sprayed with different doses of *S. frugiperda* multiple nucleopolyhedrovirus (SfMNPV).

Dose of SfMNPV ^a	Neonate-to-pupation period (d) ^b	Neonate-to-eclosion period (d) ^b	Larval weight at 12 d (mg) ^b	Pupal weight (mg) ^b	Number of eggs/female ^b
Bt soybean					
0 (control)	24.38 \pm 0.66 c	33.81 \pm 0.70 c	69.2 \pm 8.5 a	127.4 \pm 2.2 a	317.56 \pm 28.95 a
10% of field dose (7.50×10^8 OBs/L)	25.72 \pm 0.38 c	35.32 \pm 0.50 c	50.7 \pm 5.0 b	107.5 \pm 3.9 b	345.00 \pm 26.80 a
25% of field dose (1.88×10^9 OBs/L)	32.20 \pm 0.89 b	41.92 \pm 0.89 b	42.1 \pm 3.2 c	73.8 \pm 4.0 c	223.73 \pm 37.01 b
50% of field dose (3.75×10^9 OBs/L)	39.68 \pm 1.32 a	49.66 \pm 1.35 a	38.8 \pm 3.4 c	72.9 \pm 5.3 c	47.36 \pm 13.10 c
Field dose (7.50×10^9 OBs/L)	— ^c	—	—	—	—
Non-Bt soybean					
0 (control)	20.39 \pm 0.20 b	29.25 \pm 0.14 c	120.8 \pm 7.7a	160.2 \pm 4.1 a	1210.07 \pm 97.26 a
10% of field dose (7.50×10^8 OBs/L)	21.02 \pm 0.32 b	30.22 \pm 0.42 c	110.1 \pm 5.4a	157.8 \pm 5.2 a	933.29 \pm 54.22 a
25% of field dose (1.88×10^9 OBs/L)	23.17 \pm 0.52 b	33.90 \pm 0.79 b	86.9 \pm 4.4 b	100.9 \pm 4.8 b	422.12 \pm 41.77 b
50% of field dose (3.75×10^9 OBs/L)	30.12 \pm 0.62 a	42.08 \pm 0.67 a	85.4 \pm 4.6 b	68.3 \pm 4.3 c	181.05 \pm 45.22 c
Field dose (7.50×10^9 OBs/L)	— ^c	—	—	—	—

^aThe field dose of SfMNPV used against FAW neonates was 100 mL Cartugen[®]/100L water (representing 7.5×10^9 Occlusion Bodies (OBs)/L water).

^bMeans \pm SE followed by the same letter in each column and soybean are not significantly different (LSMEANS followed by Tukey test; $P > 0.05$).

^cNo insects survived to measure the biological parameter.

Table 3. Fertility life table parameters of *S. frugiperda* developing on Bt soybean leaves expressing Cry1Ac protein and non-Bt soybean sprayed with different concentrations of *S. frugiperda* multiple nucleopolyhedrovirus (SfMNPV).

Dose of SfMNPV ^a	Fertility life table parameter ^{b,c}		
	<i>T</i> (days)	<i>R</i> _o (♀/♀)	<i>r</i> _m (♀/♀*day)
Bt soybean			
0 (control)	46.36 ± 0.09 c	59.70 ± 5.44 a	0.088 ± 0.002 a
10% of field dose (7.50 × 10 ⁸ OBs/L)	45.38 ± 0.18 c	60.66 ± 4.70 a	0.091 ± 0.002 a
25% of field dose (1.88 × 10 ⁹ OBs/L)	53.70 ± 0.10 b	21.34 ± 3.53 b	0.057 ± 0.003 b
50% of field dose (3.75 × 10 ⁹ OBs/L)	59.50 ± 0.48 a	1.36 ± 0.38 c	0.006 ± 0.005 c
Field dose (7.50 × 10 ⁹ OBs/L)	— ^d	—	—
Non-Bt soybean			
0 (control)	41.43 ± 0.15 c	587.82 ± 45.78 a	0.154 ± 0.002 a
10% of field dose (7.50 × 10 ⁸ OBs/L)	41.96 ± 0.11 c	545.44 ± 23.86 a	0.140 ± 0.001 a
25% of field dose (1.88 × 10 ⁹ OBs/L)	44.95 ± 0.12 b	84.13 ± 8.33 b	0.099 ± 0.022 b
50% of field dose (3.75 × 10 ⁹ OBs/L)	53.59 ± 0.11 a	18.55 ± 2.32 c	0.055 ± 0.024 c
Field dose (7.50 × 10 ⁹ OBs/L)	— ^d	—	—

^aThe field dose of SfMNPV used against FAW neonates was 100 mL Cartugen[®]/100L water (representing 7.5 × 10⁹ Occlusion Bodies (OBs)/L water).

^b*T* = mean length of a generation (d); *R*_o = net reproductive rate (females per female per generation); and *r*_m = intrinsic rate of population increase (per day).

^cMeans ± SE followed by the same letter in each column and soybean are not significantly different (LSMEANS followed by Tukey test; *P* > 0.05).

^dNo insects survived to measure the biological parameter.

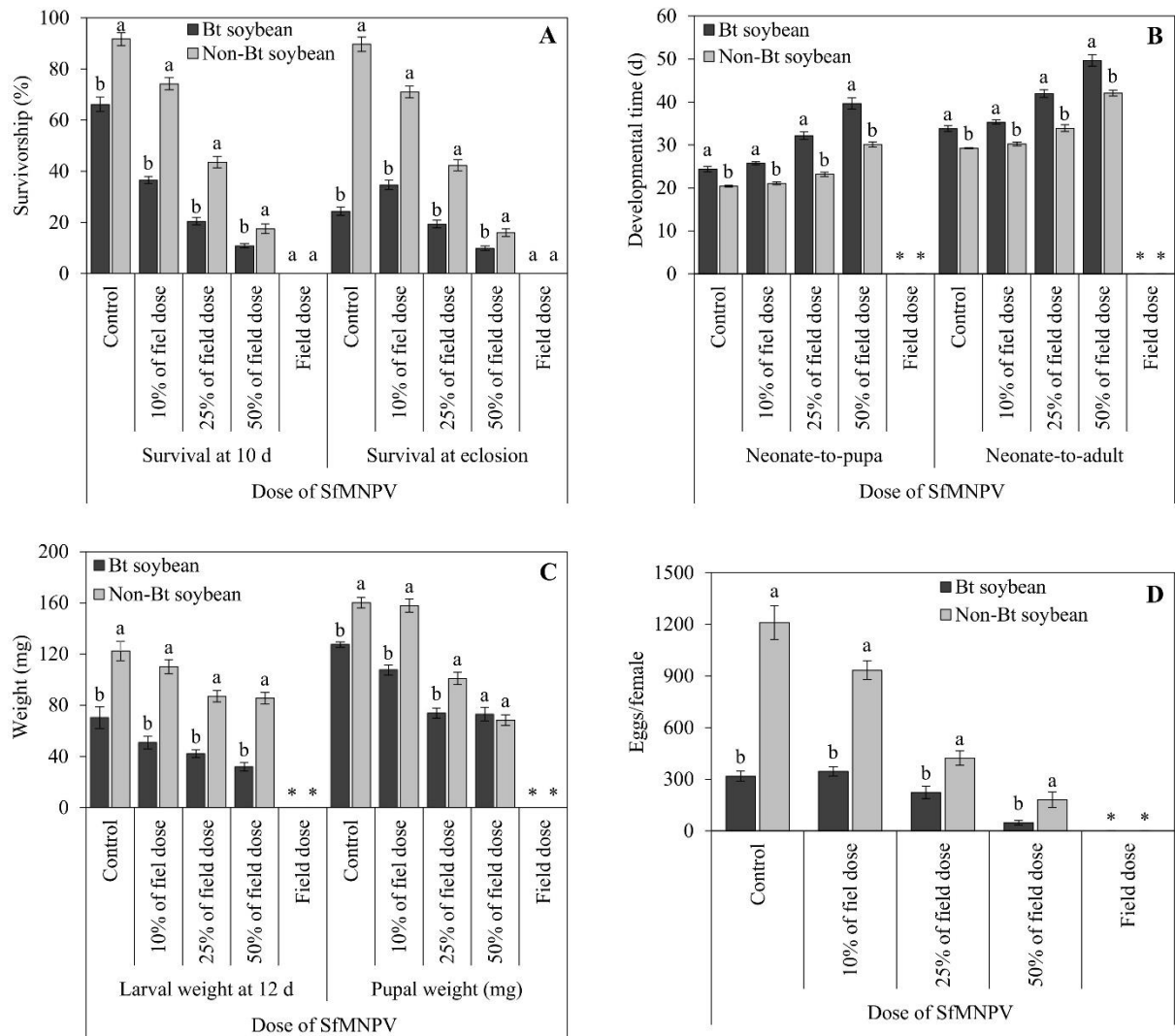


Figure 1. Survivorship (A), developmental time (B), larval and pupal weights (C) and fecundity (D) of *S. frugiperda* developing on Bt soybean expressing Cry1Ac protein and non-Bt soybean sprayed with different doses of *S. frugiperda* multiple nucleopolyhedrovirus: field dose (7.50×10^9 OBs/L), 50% of field dose (3.75×10^9 OBs/L), 25% of field dose (1.88×10^9 OBs/L), and 10% of field dose (7.50×10^8 OBs/L). Pairs of bars (\pm SE) with the same letter are not significantly different (LSMEANS followed by Tukey test; $P > 0.05$). *No insects survived to measure the biological parameter.

Table S1. *Spodoptera frugiperda* population growth parameters developing on Bt soybean expressing Cry1Ac protein and non-Bt soybean sprayed with different doses of *S. frugiperda* multiple nucleopolyhedrovirus (SfMNPV).

Dose of SfMNPV ^a	Fertility life table parameter ^{b,c}		
	<i>T</i> (days)	<i>R_o</i> (♀/♀)	<i>r_m</i> (♀/♀*day)
0 (control)			
Bt soybean	46.36 ± 0.09 a	59.70 ± 5.44 b	0.088 ± 0.002 b
Non-Bt soybean	41.43 ± 0.15 b	587.82 ± 45.78 a	0.154 ± 0.002 a
10% of field dose (7.50 × 10⁸ OBs/L)			
Bt soybean	45.38 ± 0.18 a	60.66 ± 4.70 b	0.091 ± 0.002 b
Non-Bt soybean	41.96 ± 0.11 b	352.59 ± 23.86 a	0.140 ± 0.001 a
25% of field dose (1.88 × 10⁹ OBs/L)			
Bt soybean	53.70 ± 0.10 a	21.34 ± 3.53 b	0.057 ± 0.003 b
Non-Bt soybean	44.95 ± 0.12 b	84.13 ± 8.33 a	0.099 ± 0.022 a
50% of field dose (3.75 × 10⁹ OBs/L)			
Bt soybean	59.50 ± 0.48 b	1.36 ± 0.38 b	0.006 ± 0.005 b
Non-Bt soybean	53.59 ± 0.11 a	18.55 ± 2.32 a	0.055 ± 0.024 a
Field dose (7.50 × 10⁹ OBs/L)			
Bt soybean	— ^d	—	—
Non-Bt soybean	— ^d	—	—

^aThe SfMNPV field dose used against *S. frugiperda* neonates was 100 mL Cartugen[®]/100L water (representing 7.5 × 10⁹ Occlusion Bodies (OBs)/L water).

^b*T* = mean length of a generation (d); *R_o* = net reproductive rate (females per female per generation); and *r_m* = intrinsic rate of population increase (per day).

^cMeans ± SE followed by the same letter in each column and concentration are not significantly different (*t*-test for pairwise group comparisons, *P* > 0.05).

^dNo insects survived to measure the biological parameter.

3. Considerações finais

O uso do vírus SfMNPV se mostrou eficiente no manejo de lagartas de primeiro instar larval de *S. frugiperda* em condições de laboratório, quando utilizado concentrações recomendadas pelo fabricante. Frente aos resultados, a alternativa de controle biológico com a utilização do vírus SfMNPV é considerada promissora para o manejo da lagarta do cartucho na cultura da soja Bt e não Bt. Salienta-se também a importância dos efeitos subletais do vírus que podem gerar uma supressão populacional da praga, seja na fase larval, como também na fase adulta com reduções da fecundidade dos adultos. Assim como, devido ao perfil toxicológico favorável do baculovírus SfMNPV sobre a espécie-alvo e a ampla seletividade sobre inimigos naturais benéficos, sua utilização se torna oportuna para o estabelecimento de programas de MIP e MRI. Entretanto, novos estudos devem ser realizados em situação de campo para determinar as melhores condições climáticas para a aplicabilidade na cultura, uma vez com que, a radiação solar, a temperatura, a umidade, podem influenciar diretamente na eficiência e na estabilidade do vírus.

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