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The Biological Activity of an *Sf*MNPV-Based Biopesticide on a Resistant Strain of *Spodoptera frugiperda* Developing on Transgenic Corn Expressing Cry1A.105 + Cry2Ab2 + Cry1F Insecticidal Protein

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Abstract: Insecticides based on baculoviruses have become an alternative for pest control in different agricultural crops. The aim of this study was to assess the biological activity of the bioinsecticide Cartugen™ (*Sf*MNPV: Baculoviridae: Alphabaculovirus) on larvae of *Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae) resistant to Bt corn expressing the insecticidal proteins Cry1A.105+Cry2Ab2+Cry1F. In addition, we assessed the efficiency of *Sf*MNPV on *S. frugiperda* control in the field from natural infestation of the pest during two agricultural seasons. The results showed that no larvae survived 10 days after being inoculated with Bt and non-Bt corn leaves contaminated with 1.50×10^{10} occlusion bodies (OBs)/L (equivalent to the recommended dose of the product). However, when using doses equivalent to 50% (7.50×10^{9} OBs/L) and 25% (3.75×10^{9} OBs/L), the larval mortality ranged from 21.12% to 46.55%, respectively. Although larvae resistant to the Cry1A.105+Cry2Ab2+Cry1F proteins, when exposed to 50% of the *Sf*MNPV dose (7.50×10^{9} OBs/L), showed reductions in larval weight (52 to 67% reduction), pupal weight (32 to 59% reduction), and total fecundity (67 to 86% reduction) compared to the control. Furthermore, doses above 25% (3.75×10^{9} OBs/L^{-1}) caused a population decrease in the growth of the species in both Bt and non-Bt corn according to the fertility life table. In the field, at 7 and 10 DAA (days after application), corn plants sprayed with *Sf*MNPV (1.50×10^{10} OBs/L) showed reductions in leaf damage according to the Davis scale. However, from 14 to 21 DAA, there was an increase in leaf damage in corn leaves from both treatments, with or without the application of *Sf*MNPV. This shows that *Sf*MNPV may be an important strategy in the integrated management and resistance management of *S. frugiperda*.

Keywords: fall armyworm; baculovirus; *Bacillus thuringiensis*

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1. Introduction

Brazil is one of the world's leading corn producers with a cultivated area of approximately 25 million hectares [1], 85% of which is cultivated with Bt corn technologies, i.e., corn plants that express insecticidal proteins based on *Bacillus thuringiensis* in the plant tissue. Despite the availability of various Bt corn technologies for commercial cultivation in Brazil, the majority express insecticidal proteins from the Cry1 group [2], which has increased selection pressure in the management of the fall armyworm *Spodoptera frugiperda* (J.E Smith, 1797) (Lepidoptera: Noctuidae), which is considered one of the major corn pests worldwide [3]. This high selection pressure favored the caterpillar's survival on different

Bt corn technologies, such as corn plants expressing the Cry1F protein [4], Cry1Ab [5], Cry1A.105+Cry2Ab2 [2], and Cry1A.105+Cry2Ab2+Cry1F [6].

Given this scenario, alternative strategies for the management of the fall armyworm should be studied since, in Brazil, the species has also evolved resistance to different groups of synthetic insecticides, such as lambda-cyhalothrin [7], chlorpyrifos [8], spinosad [9] and diamides [10] and recently by emamectin benzoate [11]. Within this context of insect resistance management (IRM), the use of biopesticides based on entomopathogenic viruses can be a sustainable alternative to be incorporated into control programmes [12], especially with advances in the production and formulation of baculovirus-based products [12,13].

In the 1980s, the use of the baculovirus *Anticarsia gemmatalis* Multiple NPV (*Ag*MNPV) in soybean was widely recognized for its success in the biological management of the insect. The *Ag*MNPV baculovirus has been applied to over a million hectares a year to control *Anticarsia gemmatalis* Hübner (Lepidoptera: Erebidae) [12]. However, the use of *Ag*MNPV has decreased over time due to the advent of chemical insecticides that are highly effective at controlling them [14]. Baculovirus-based insecticides have had their biological activity reported for different agricultural pests, including *Helicoverpera armigera* (Hübner) (Lepidoptera: Noctuidae) in cotton [14], *S. frugiperda* in corn and soybean [15], and *A. gemmatalis* and *Chrysodeixes includens* (Walker, (1858)) (Lepidoptera: Noctuidae) on soybean [16].

In Brazil, with the promising adoption of the entomopathogenic virus Cartugen™ (*Sf*MNPV: Baculoviridae: Alphabaculovirus)—an insecticide with a new mode of action (Group 31, Insecticide Resistance Action Committee—IRAC), registered for use in the management of *S. frugiperda* in corn, it could be an effective alternative for managing *S. frugiperda* resistance to Bt corn technologies. Furthermore, there are no reports of cross-resistance between the entomopathogen and chemical insecticides, presenting a high potential for use in IRM programs [17–20].

The aim of this study was to assess the biological activity of *Sf*MNPV-based biopesticides on *S. frugiperda* strains susceptible and resistant to Bt and non-Bt corn, as well as the efficiency of *S. frugiperda* control in the field using *Sf*MNPV.

2. Materials and Methods

2.1. Insects

The strain of *S. frugiperda* resistant to PowerCore™ technology that expresses the insecticidal proteins Cry1A.105+Cry2Ab2+Cry1F (Res strain) [6] was obtained from the Biology Laboratory of the Federal University of Pelotas. The resistant strain (Res strain) has been maintained for more than 40 generations under selection pressure on its natural food (corn leaves expressing the above-mentioned insecticidal proteins) in bioassay plates with 16 cells (5.5 cm long × 4.0 cm deep × 3.0 cm high per well) [(Advento do Brasil, São Paulo, Brazil)] on a nongelled, agar–water mixture (2.5%) until the pupal stage. The pupae are then removed and placed in Petri dishes (12 cm diameter × 1.5 cm height) lined with filter paper moistened with distilled water and placed in cylindrical PVC cages (24.0 cm height × 14.5 cm diameter) for the emergence of the adults. After emergence, the adults are transferred to cylindrical PVC cages (24.0 cm high × 14.5 cm in diameter), lined internally with newspaper, and closed at the top with thin voile fabric. The adults' food consists of a 10% aqueous solution of honey supplied via capillarity through hydrophilic cotton wool. Every 2 days, the eggs are collected and placed in plastic containers (500 mL) containing filter paper moistened with distilled water and incubated in an air-conditioned chamber (temperature 27 ± 1 °C, relative humidity 60 ± 10%, and photoperiod 14 h). After hatching, the larvae are transferred to natural food (Bt corn), to maintain selection pressure, and returned to the maintenance brood.

2.2. Biological Activity of the Insecticide SfMNPV on S. frugiperda in the Laboratory

For the bioassays, the commercial product Cartugen™ (active ingredient *Sf*MNPV, concentration 7.50×10^9 occlusion bodies (OBs)/mL) (AgBiTech, Fort Worth, TX, USA) was

used. At phenological stage V4, Bt corn plants of PowerCore™ technology and the respective non-Bt isolate were grown in 10-liter plastic pots (one plant/pot) containing soil and plant substrate in a 1:1 ratio and sprayed with the commercial product containing *Sf*NPV diluted in water at the following doses (treatments = T): T0 (control—unsprayed corn plants); T1: 1.50×10^{10} occlusion bodies (OBs)/L), equivalent to 100% of the recommended field dose (100 mL of Cartugen™/100 L of water); T2: 7.50×10^{9} OBs/L (equivalent to 50% of the recommended dose); T3: 3.75×10^{9} OBs/L (equivalent to 25% of the recommended dose), and T4: 1.50×10^{9} OBs/L (equivalent to 10% of the recommended dose). The different doses of *Sf*MNPV were applied using a 10-L capacity knapsack sprayer equipped with a cone nozzle (XR 110.02 fan nozzle tips). After four hours, corn leaves (Bt and non-Bt) were removed and transported to the laboratory. The leaves were then placed on a gelled 2.0% agar–water mixture in 16-well plastic plates (Advento do Brasil, São Paulo, Brazil). Subsequently, each well was infested with one larva (<24 h) of *S. frugiperda* according to each treatment. The experimental design was entirely randomized with 10 repetitions (plates) of 16 neonates per treatment (n = 160). Afterwards, the plates were closed and placed in a chamber at 27 ± 1 °C, 60 ± 10% RH and 14 h photoperiod. The corn leaves were replaced every 48 h throughout the larval period with leaves obtained from the same plants initially used in the bioassays. The biological parameters assessed were larval survival at 10 days, duration of the larval and biological cycle periods (larva to adult), larval weight (mg) at 12 days after inoculation, and pupal weight (mg) 24 h after pupation and sex ratio. In the adult stage, eight to 15 couples/treatment were kept in PVC cages (23 cm high × 15 cm in diameter) lined internally with white paper and closed with transparent fabric. The oviposition period (days), longevity (days), and total fecundity (number of eggs per female over the longevity period) were then assessed.

2.3. Biological Activity of the Insecticide SfMNPV on S. frugiperda in the Field

Field experiments were carried out in the municipality of Arroio Grande, Rio Grande do Sul, Brazil (32° 14′ 19″ S; 53° 5′ 27″ O), during the 2020/2021 and 2021/2022 harvest. The treatments consisted of Bt PowerCore™ corn expressing the Cry1A.105+Cry2Ab2+Cry1F proteins and non-Bt corn isoline. The experimental design was entirely randomized, with four replications (plots) per corn isoline. The experimental plots comprised four rows of corn, 4.0 m long, with a row spacing of 0.80 m. Bt and non-Bt corn seeds were sown manually at a density of four seeds/linear meter. The experimental area is characterized by a high history of natural infestation by *S. frugiperda*. In order to monitor the level of infestation of the pest on the corn plants, after emergence, the plants were evaluated every 3 days by analyzing three central leaves of the husk of 10 corn plants from the central line of the plot. The corn plants evaluated were marked with a strip of yellow tape (5 cm long) near the base of the plant. The Cartugen™ baculovirus (200 mL of the commercial product per 150 L of water) was applied when 10% of the plants quantified in each sample had a damage score > 3 (phenological stage V4), according to the scale of Davis [21]. The number of plants with a damage score > 3 is suggested as a criterion for the use of insecticides against *S. frugiperda* in Brazil. The baculovirus was applied using a costal sprayer pressurized with CO_2, with a single nozzle (XR 110.02 fan tip), applying a spray volume of 150 L/ha. Experimental plots without baculovirus application were used as a control treatment. After application, the damage caused by *S. frugiperda* to the corn plants was assessed at 0 (before application), 3, 7, 10, 14 and 21 DAA (days after application). For each evaluation date, a damage score was assigned to each plant (10 plants per plot as described above) according to the Davis scale [21].

2.4. Statistical Analyses

To assess the biological activity of *Sf*MNPV on *S. frugiperda* on Bt and non-Bt corn, data from all biological parameters evaluated were subjected to a two-way ANOVA using the PROC GLM. Factor A was represented by one Bt corn plant (expressing Cry1A.105+Cry2Ab2+Cry1F) and one non-Bt corn plant. Factor B was composed of four doses of

*Sf*MNPV-based product (100%, 50%, 25%, and 10% of the field recommended rate) and untreated controls. Corn, doses of *Sf*MNPV, and interactions were used as fixed factors in the model. Mean differences were estimated by least-square means (LSMEANS option of PROC GLM) using a Tukey–Kramer adjustment test ($p < 0.05$).

Based on the duration of the developing period from egg to adult, sex ratio, fecundity and egg-to-adult survival (%), life table parameters were estimated for each treatment. The original data for all individuals were analyzed according to the theoretical model proposed by Chi & Liu [22] using the TWOSEXMSChart 2021 program (http://140.120.197.173/ecology/Download/TWOSEX-MSChart.rar accessed on 23 April 2024) [23]. For each treatment, the following parameters were estimated:

The net reproduction rate (R_o):

$$R_o = \sum_{x=0}^{\infty} l_x m_x \quad (1)$$

The intrinsic rate of increase (r):

$$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1 \quad (2)$$

The mean generation time (T):

$$T = \ln R_o / r \quad (3)$$

and the finite rate of increase (λ):

$$\lambda = e^r \quad (4)$$

The mean and standard error (SE) of each parameter were estimated by the bootstrap method [24]. During the bootstrap procedure, the data for each population parameter were resampled 40,000 times. The means for each treatment were compared by a paired bootstrap test based on the difference confidence interval [24].

To verify the effect of baculovirus in Bt corn and non-Bt corn on the *S. frugiperda* population in the field at different times (days after application—DAA), the data were subjected to a repeated-measures analysis using generalized linear mixed models (GLMM) of the "lme4" package [25] with a binomial distribution. For this purpose, the effects of explanatory characteristics (treatments) and time were considered as fixed factors, while the repeated measures in each corn plant in time were considered random. The effects of treatment and time were assessed by likelihood-ratio tests ($p < 0.05$) between a full model and a reduced model. The same test was used to verify the significance of the interaction of treatment with time, comparing a model with the interaction and a model without the interaction. Furthermore, for each time, generalized linear models (GLM) with quasi-Poisson distribution were used to analyze the count data of leafhoppers on the corn plants. For the variables analyzed, goodness-of-fit was assessed through half-normal plots with simulation envelopes using the "hnp" package. In case of significant differences among treatments, multiple comparisons with the Tukey test ($p < 0.05$) were performed using the "glht" function of the "multicomp" package, with adjusted p values. When significant differences between treatments were detected, the data were submitted to the t-test at a 5% significance level. All analyses were performed using the statistical software "R", version 4.2.2.

3. Results

3.1. Survival

The main effects of *Sf*MNPV dose, corn, and corn × *Sf*MNPV dose were all significant for neonate survival at 10 d.p.i. ($F = 243.00$; $df = 4, 90$; $p < 0.0001$; $F = 211.33$; $df = 1, 90$; $p < 0.0001$; $F = 99.11$; $df = 4, 90$; $p < 0.0001$, respectively). There were no survivors beyond 10 d.p.i on Bt and non-Bt corn leaves sprayed with the field dose of *Sf*MNPV (Table 1).

However, there was high survival (85.3% ± 2.75) of the Res strain on Bt corn in the absence of *Sf*MNPV, demonstrating the strain's resistance to corn technology (Table 1). In contrast, at the lowest dose of *Sf*MNPV (10% of field dose or 1.50×10^9 OBs/L), there was the highest survival of neonates to the adult stage of 88.1% (Bt corn) and 84.1% (non-Bt corn) of the larvae, being statistically similar to the control treatment (Table 1).

Table 1. Survivorship of *S. frugiperda* neonates developing on Bt corn expressing Cry1A.105+ Cry2Ab2+Cry1F protein and non-Bt corn sprayed with different doses of *S. frugiperda* multiple nucleopolyhedrovirus (*Sf*MNPV).

Dose of *Sf*MNPV [a]	Survivorship at 10 d.p.i. (%) [b]	Survivorship at Adult Eclosion (%) [b]
Bt corn		
0 (control)	85.32 ± 2.75 a	90.11 ± 1.70 a
10% of field dose (1.50×10^9 OBs/L)	71.34 ± 2.14 a	88.14 ± 1.90 a
25% of field dose (3.75×10^9 OBs/L)	36.11 ± 2.89 b	55.11 ± 1.10 b
50% of field dose (7.50×10^9 OBs/L)	21.12 ± 1.76 c	21.14 ± 2.06 c
Field dose (1.50×10^{10} OBs/L)	0.00 ± 0.00 d	0.00 ± 0.00 d
Non-Bt corn		
0 (control)	96.30 ± 1.78 a	90.11 ± 2.65 a
10% of field dose (1.50×10^9 OBs/L)	81.10 ± 1.12 a	84.01 ± 1.76 a
25% of field dose (3.75×10^9 OBs/L)	43.32 ± 1.20 b	35.12 ± 1.18 b
50% of field dose (7.50×10^9 OBs/L)	22.31 ± 2.11 c	11.07 ± 1.23 c
Field dose (1.50×10^{10} OBs/L)	0.00 ± 0.00 d	0.00 ± 0.00 d

[a] The field dose of *Sf*MNPV used against *S. frugiperda* neonates was 100 mL Cartugen™/100 L water. [b] Means ± SE with the same letter in each column and corn are not significantly different (LSMEANS followed by the Tukey test; $p > 0.05$).

3.2. Development of the Immature Phase

Neonates of *S. frugiperda* surviving on Bt and non-Bt corn when fed corn leaves with the presence of 50% of the field dose (7.50×10^9 OBs/L) of *Sf*MNPV showed an extension of the period from neonate to adult (43 days) in relation to the control (29 days) and with the lowest dose evaluated (10% field dose—1.50×10^9 OBs/L) in both Bt and non-Bt corn without or with the presence of the baculovirus) (Table 2). In addition, *S. frugiperda* neonates exposed to *Sf*MNPV at doses of 25 (3.75×10^9 OBs/L) and 50% (7.50×10^9 OBs/L) the field dose showed a significant reduction in larval weight (Table 2), and at 50% of the dose the reduction in larval weight was over 60% when compared to the control treatment (Table 2). In addition, pupae from larvae fed on Bt corn leaves (32% reduction) and non-Bt corn leaves (51% reduction) contaminated with 50% of the *Sf*MNPV dose showed a lower pupal weight compared to the control treatment (Table 2). Based on the chi-squared test values, there were no significant differences in the sex ratio between the treatments (Table 2). However, at 25 and 50% of the *Sf*MNPV dose, *S. frugiperda* females had a shorter oviposition period (variation between 5.3 and 5.5 days) and were less long-lived (variation between 5.0 and 5.9 days) compared to the control treatment (oviposition period of 7.1 days and female longevity of 8.2 days) (Table 2). In addition, resistant *S. frugiperda* larvae surviving on Bt and non-Bt corn sprayed with 50% and 25% of the field dose of *Sf*MNPV gave rise to females with the lowest total fecundity rates compared to the control treatment (Table 2), with the 50% dose of *Sf*MNPV reducing oviposition by 67% in Bt corn and 86% in non-Bt corn (Table 2). In contrast, females from larvae fed on corn leaves contaminated with 10% of the *Sf*MNPV dose showed an average fecundity similar to the control treatment in both Bt and non-Bt corn (Table 2).

Table 2. Biological parameters of *S. frugiperda* developing on Bt corn expressing Cry1A.105+ Cry2Ab2+Cry1F protein and non-Bt corn sprayed with different doses of *S. frugiperda* multiple nucleopolyhedrovirus (*Sf*MNPV).

Dose of *Sf*MNPV [a]	Neonate-to-Adult Period (d) [b]	Larval Weight at 12 d (mg) [b]	Pupal Weight (mg) [b]	Sex Rate	Oviposition [b]	Longevity (Days)[b]	Number of Eggs/Female [b]
Bt corn							
0 (control)	29.11 ± 1.05 c	102.1 ± 0.012 a	119.0 ± 0.012 a	0.51 [ns]	7.1 ± 0.54 a	8.2 ± 0.65 a	291.50 ± 13.11 a
10% of field dose (1.50 × 10^9 OBs/L)	29.71 ± 1.30 c	100.0 ± 0.011 a	108.0 ± 0.017 a	0.44	7.9 ± 0.33 a	8.1 ± 0.89 a	281.00 ± 12.11 a
25% of field dose (3.75 × 10^9 OBs/L)	36.21 ± 1.09 b	70.0 ± 0.011 b	102.0 ± 0.011 a	0.49	5.5 ± 0.87 b	5.9 ± 0.95 b	145.73 ± 27.02 b
50% of field dose (7.50 × 10^9 OBs/L)	43.71 ± 1.11 a	39.0 ± 0.012 c	81.0 ± 0.012 b	0.43	5.3 ± 0.95 b	5.4 ± 0.56 b	98.30 ± 11.10 c
Field dose (1.50 × 10^{10} OBs/L)	– [c]	–	–	-	-		–
Non-Bt corn							
0 (control)	29.80 ± 0.20 b	119.8 ± 0.11 a	154.0 ± 0.012 a	0.45 [ns]	7.9 ± 0.87 a	7.9 ± 0.89 a	298.11 ± 15.26 a
10% of field dose (1.50 × 10^9 OBs/L)	30.02 ± 0.31 b	109.0 ± 0.012 a	145.0 ± 0.012 a	0.52	7.1 ± 0.45 a	8.4 ± 0.95 a	310.04 ± 50.22 a
25% of field dose (3.75 × 10^9 OBs/L)	31.27 ± 0.32 b	81.0 ± 0.012 b	125.0 ± 0.014 a	0.49	5.4 ± 0.89 b	5.0 ± 0.87 b	121.00 ± 25.77 b
50% of field dose (7.50 × 10^9 OBs/L)	42.23 ± 0.32 a	40.0 ± 0.014 c	64.0 ± 0.014 b	0.45	5.3 ± 0.76 b	5.6 ± 0.66 b	42.05 ± 21.22 c
Field dose (1.50 × 10^{10} OBs/L)	– [c]	–	–	-	-	-	–

[a] The field dose of *Sf*MNPV used against *S. frugiperda* neonates was 100 mL Cartugen™/100 L water. [b] Means ± SE with the same letter in each column and corn are not significantly different (LSMEANS followed by the Tukey test; $p > 0.05$). [c] No insects survived to measure the biological parameter. [ns]: not significant by the chi-square test.

3.3. Fertility Life Table

According to the results. *S. frugiperda* females from resistant larvae fed corn leaf in the presence of 50% of the *Sf*MNPV dose produced 30 females/newborn female/generation (R_o) in 60 days (T). In contrast, the survivors of the progeny at the lowest doses (10% of the recommended dose—1.50 × 10^9 OBs/L) produced an average of 240 females/female in 42 to 43 days, which did not differ from the control (Table 3). In addition, females from the treatment with 50% *Sf*MNPV had a natural population increase rate (r_m) of less than 0.0044 (Bt corn) and 0.062 (non-Bt corn, indicating an average 50% lower capacity for population increase compared to the control treatment and with the use of 10% *Sf*MNPV (Table 3).

Table 3. Fertility life table parameters of *S. frugiperda* developing on Bt corn leaves expressing Cry1A.105+Cry2Ab2+Cry1F protein and non-Bt corn sprayed with different concentrations of the *S. frugiperda* multiple nucleopolyhedrovirus (*Sf*MNPV).

Dose of *Sf*MNPV [a]	Fertility Life Table Parameter [bc]		
	***T* (Days)**	***R_o* (♀/♀)**	***r_m* (♀/♀× Dias)**
Bt corn			
0 (control)	43.11 ± 0.03 c	261.11 ± 3.41 a	0.090 ± 0.002 a
10% of field dose (1.50 × 10^9 OBs/L)	39.25 ± 0.14 c	245.54 ± 4.21 a	0.081 ± 0.002 a
25% of field dose (3.75 × 10^9 OBs/L)	56.11 ± 0.11 b	45.11 ± 2.51 b	0.065 ± 0.003 b
50% of field dose (7.50 × 10^9 OBs/L)	60.50 ± 0.41 a	31.10 ± 0.41 c	0.044 ± 0.001 c
Field dose (1.50 × 10^{10} OBs/L)	– [d]	–	–
Non-Bt corn			
0 (control)	42.21 ± 0.15 c	298.82 ± 35.74 a	0.131 ± 0.001 a
10% of field dose (1.50 × 10^9 OBs/L)	40.92 ± 0.11 c	242.44 ± 33.11 a	0.134 ± 0.002 a
25% of field dose (3.75 × 10^9 OBs/L)	45.10 ± 0.11 b	78.13 ± 8.32 b	0.092 ± 0.022 b
50% of field dose (7.50 × 10^9 OBs/L)	59.46 ± 0.13 a	30.32 ± 2.12 c	0.062 ± 0.014 c
Field dose (1.50 × 10^{10} OBs/L)	– [d]	–	–

[a] The field dose of *Sf*MNPV used against neonates of *S. frugiperda* was 100 mL Cartugen™/100 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). [c] Means ± SE within a column followed by the same letter are not significantly different (*t*-test for pairwise group comparisons. $p > 0.05$). [d] No insects survived to measure the biological parameter.

3.4. Efficiency of SfMNPV on S. frugiperda Control in the Field

At 3 DAA, the damage scores on corn plants, according to the Davis scale, remained constant and the same as observed in the previous evaluation for both harvests for Bt and non-Bt corn plants with or without baculovirus application (Table 4). However, in the evaluation carried out at 7 DAA, there was a reduction in damage to the leaves of Bt and non-

Bt corn in the two crops evaluated when Cartugem™ was used in the experimental plots (Table 4). In contrast, in the experimental plots in which the application of the Cartugen™ baculovirus was not used, the damage scores on the corn plants were significantly higher compared to the evaluation carried out on the third day. At 10 DAA, in the experimental plots cultivated with Bt corn plants with the application of the Cartugen™ baculovirus, the damage scores were the same as the scores assigned at 7 DAA, demonstrating that there was no increase in leaf damage caused by *S. frugiperda* larvae (Table 4). In contrast, corn plants (Bt and non-Bt) without the presence of Cartugen™ showed a significant increase in damage scores. However, at 14 and 21 DAA, all the corn plants evaluated (Bt and non-Bt corn) with or without the application of Cartugen™ showed a significant increase in damage caused by *S. frugiperda* when compared to 10 DAA (with or without the application of Cartugen™) (Table 4).

Table 4. Mean of damage scores Davis et al. scale [21] caused by *Spodoptera frugiperda* larvae (natural infestation) in corn crops treated with *Spodoptera* baculovirus (Cartugen™).

Host	Treatment	Pre-Sampling	Days after Application				
		0 [a]	3 [a]	7 [a]	10 [a]	14 [a]	21 [a]
2020/2021 Harvest							
Bt corn	WITHOUT application of Cartugen™	2.8 ± 0.41 Aa	3.0 ± 0.22 Aa	4.5 ± 0.36 Ba	5.6 ± 0.26 Ca	6.6 ± 0.32 Da	6.6 ± 0.33 Da
Bt corn	WITH application of Cartugen™	2.6 ± 0.42 Aa	2.9 ± 0.17 Aa	1.0 ± 0.39 Bb	1.7 ± 0.26 Bb	3.3 ± 0.33 Cb	4.2 ± 0.32 Cb
Non-Bt corn	WITHOUT application of Cartugen™	4.1 ± 0.37 Aa	4.4 ± 0.26 Aa	5.6 ± 0.29 Ba	5.9 ± 0.34 Ba	7.2 ± 0.29 Ca	7.6 ± 0.22 Ca
Non-Bt corn	WITH application of Cartugen™	4.4 ± 0.54 Aa	4.7 ± 0.13 Aa	1.5 ± 0.37 Bb	2.0 ± 0.39 Bb	4.8 ± 0.24 Cb	5.0 ± 0.14 Cb
2021/2022 Harvest							
Bt corn	WITHOUT application of Cartugen™	2.1 ± 0.40 Aa	2.7 ± 0.26 Aa	4.7 ± 0.45 Ba	6.6 ± 0.13 Ca	6.8 ± 0.17 Ca	7.9 ± 0.32 Da
Bt corn	WITH application of Cartugen™	2.5 ± 0.33 Aa	3.0 ± 0.27 Aa	2.2 ± 0.39 Bb	2.7 ± 0.10 Bb	4.3 ± 0.34 Cb	5.2 ± 0.12 Cb
Non-Bt corn	WITHOUT application of Cartugen™	2.0 ± 0.31 Aa	3.2 ± 0.21 Aa	5.3 ± 0.26 Ba	6.9 ± 0.16 Ca	7.4 ± 0.11 Da	8.1 ± 0.20 Da
Non-Bt corn	WITH application of Cartugen™	2.4 ± 0.32 Aa	2.9 ± 0.16 Aa	1.8 ± 0.23 Bb	2.3 ± 0.22 Bb	4.8 ± 0.44 Cb	5.0 ± 0.19 Cb

[a] Means ± SE followed by the same uppercase letters within the line do not differ significantly from each other (GLM with quasi-binomial distribution followed by Tukey's posthoc test at 5%). When significant differences between treatments (WITHOUT application of Cartugen™ and WITH application of Cartugen™), "lowercase letters" were detected within each corn technology, and the data were submitted to the *t*-test at a 5% significance level.

4. Discussion

This study is considered the first to report the control efficiency of a resistant strain of *S. frugiperda* to a Bt corn technology in Brazil using a biopesticide based on *Sf*MNPV. The results showed that *S. frugiperda* neonates resistant to Bt corn technology exposed to the recommended field dose of *Sf*MNPV did not survive beyond 10 days (see Table 1). This made it clear that the combination of currently available Bt corn and baculovirus-based products can be considered an ecologically safe and selective tactic against resistant *S. frugiperda* strains that survive on Bt corn expressing the Cry1A.105+Cry2Ab2+Cry1F proteins. In addition, *S. frugiperda* neonates exposed to lower doses (50% and 25% of the field dose) of *Sf*MNPV applied to Bt and non-Bt corn showed sublethal effects through prolonged development time of the larva-pupa and larva-adult periods reduced larval and pupal weights and reduced fecundity. Lethal and sublethal pathogenic effects of baculovirus-based insecticides containing nucleopolyhedrovirus (NPVs) have previously been reported in other *Spodoptera* species. Reductions in fecundity were verified in *Splt*MNPV (*Spodoptera*

litura MNPV) infections in *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) [26], lower fecundity, altered sex ratio, and longer larval and pupal phases in *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) infected by *S. exigua* MNPV (*Se*MNPV) [27]. Sublethal infections by NPVs of other lepidopteran pests were also related to lower fecundity and longer larval and pupal periods in *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) and fewer females produced per female in *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae) [16].

The sublethal effects are associated with a marked characteristic of the baculovirus in reducing the metabolism of glycogen total lipids and soluble proteins [28,29]. The reduction in metabolism leads to lower food consumption by contaminated insects and, consequently, reductions in growth and development capacity in the immature and adult stages [17,20]. According to Matthews et al. [30], this loss of the pest's biotype potential can be explained by a combination of physiological morphological and behavioral factors. According to the authors, adults from larvae contaminated with baculoviruses suffer an interruption in vitellogenesis, resulting in the reabsorption of the developing egg, providing lower fecundity [31–34]. In addition, contaminated insects can show changes in sexual behavior and in the production of sex pheromones, especially in the attraction between males and females, resulting in a lower mating rate, which impacts the population dynamics of the species [15,16,32,35].

By analyzing the data from the fertility life table, it is possible to see that the sublethal effects do not only occur in insects that have been directly contaminated with *Sf*MNPV but also in their descendants due to the fact that there are transgenerational consequences as verified in previous studies [16,27]. This is a result of the combination of two biological factors, the prolongation of the biological cycle (T-time) and the reproduction rate of the species (Ro). The combination of these two biological growth parameters led to a significant reduction in the number of annual generations of the pest, especially in insects fed corn leaves containing 50% of the recommended dose of the bioinsecticide.

In the field, using the manufacturer's recommended dose of *Sf*MNPV (1.50×10^{10} occlusion bodies (OBs)/L or 100 mL of Cartugen™/100 L of water), it was found that the baculovirus provided a significant reduction in damage to Bt and non-Bt corn leaves after application of the product. However, the effectiveness of control by reducing damage to corn leaves (Davis scale) was up to 10 DAA. According to this result, it was clear that the product needed to be reapplied after this evaluation date. One of the factors that negatively affects the efficiency of baculovirus-based products in pest control is the action of ultraviolet rays [17]. According to Martínez et al. [36], the persistence of baculovirus-based products in the field can be as little as 24 h or up to 12 days after application. The product's high susceptibility to degradation by sunlight is one of the factors behind its low persistence in the field [17]. Different studies have shown that applying products with relative humidity above 70% and at night can favor a longer average product life [27]. Natural selection and the isolation of materials that are resistant to light degradation have also led to greater residual activity in the field [37]. Similarly, simple additions of protective ingredients to ultraviolet degradation in the spray tank and the adoption of encapsulation technologies to extend activity in the field [37] are tools that can be adopted to improve the persistence of products.

In the study, the reduction in damage caused by *S. frugiperda* by the application of *Sf*MNPV to Bt and non-Bt corn up to 10 DAA may be associated with the high humidity conditions (>80%) during the execution of the work, associated with the application of the treatments at night (between 6 and 8 pm). The relative humidity of 80% or more at the study site was characterized by the occurrence of precipitation (on average 20 mm of rain) every three days, which may have triggered an ideal condition for the maintenance and active persistence of *Sf*MNPV on corn leaves.

According to the results obtained, the combination of Bt corn and baculovirus-based products was considered a viable strategy for the management of *S. frugiperda* in PowerCore™ corn since *S. frugiperda* has already evolved resistance to this corn technology in Brazil [6]. In addition, studies carried out by Bentivenha et al. [38] found that Brazilian

populations of *S. frugiperda* showed similar susceptibility to *Sf*MNPV and did not show cross-resistance with chemical insecticides and Bt proteins. Therefore, the adoption of biological control agents with the use of bioinsecticides containing baculoviruses can complement the control of *S. frugiperda* [39,40]. In addition, providing a new control agent with a different mode of action to be increased in integrated pest management [40] and insect resistance management (IRM) programs in Brazil [4,6], especially for *S. frugiperda*, where the pest's resistance to Bt toxins is a worrying factor for the corn crop.

5. Conclusions

(i) *Spodoptera frugiperda* resistant to Bt corn technology that expresses the insecticidal proteins Cry1A.105+Cry2Ab2+Cry1F were highly susceptible to the baculovirus *Sf*MNPV; (ii) doses of 50% (7.50×10^9 OBs/L) and 25% (3.75×10^9 OBs/L) of the recommendation caused sublethal effects in *Spodoptera frugiperda* resistant to Cry1A.105+Cry2Ab2+Cry1F; (iii) the recommended dose of *Sf*MNPV 1.50×10^{10} occlusion bodies (OBs)/L provided 100% mortality of *S. frugiperda* larvae in Bt and non-Bt corn; and (iv) the residual effect of the *Sf*MNPV-based product in the field was 10 days after application.

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Conflicts of Interest: F.C.S.G., L.N.M., I.E.d.F.M., L.F.M., W.L.F., V.N.S., J.d.B.P., A.P.S.A.S.d.R. and D.B. declare no potential conflicts of interest.

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