REVISTA CONTRIBUCIONES A LAS CIENCIAS SOCIALES

Simplified and cool texturing diets for *in vivo* inoculation of *Spodoptera frugiperda multiple nucleopolyhedrovirus* (SfMNPV)

Dietas com texturas simplificadas e frias para inoculação *in vivo* de *Spodoptera frugiperda multiple nucleopolyhedrovirus* (SfMNPV)

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Lorena Contarini Machado
PhD in Agronomy
Institution: Universidade Federal do Espírito Santo (UFES)
Address: Alegre - ES, Brasil
E-mail: contarini.machado@gmail.com

Hugo José Gonçalves dos Santos Junior
PhD in Agricultural Entomology
Institution: Universidade Federal do Espírito Santo (UFES)
Address: Alegre - ES, Brasil
E-mail: hugo.goncalves@ufes.br

Carlos Eduardo Costa Paiva
PhD in Plant Production
Institution: Universidade Federal do Espírito Santo (UFES)
Address: Alegre - ES, Brasil
E-mail: cecostapaiva@gmail.com

Julianne Almeida Rodrigues
PhD in Agronomy
Institution: Universidade Federal do Espírito Santo (UFES)
Address: Alegre - ES, Brasil
E-mail: julianne_ar@hotmail.com

Nathália Puppim de Oliveira Benachio
Graduated in Agronomy
Institution: Universidade Federal do Espírito Santo (UFES)
Address: Alegre - ES, Brasil
E-mail: nath.puppim@hotmail.com

Hugo Bolsoni Zago
PhD in Agricultural Entomology
Institution: Universidade Federal do Espírito Santo (UFES)
Address: Alegre - ES, Brasil
E-mail: hugozago@gmail.com

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Laura Vaillant Ribeiro Mauri
Master in Plant Production
Institution: Universidade Federal do Espírito Santo (UFES)
Address: Alegre - ES, Brasil
E-mail: vaillant.lr@gmail.com

Stella Arndt
Graduated in Agronomy
Institution: Universidade Federal do Espírito Santo (UFES)
Address: Alegre - ES, Brasil
E-mail: stella.arndt@edu.ufes.br

ABSTRACT
The inoculation of viral entomopathogens in vivo requires study of each pathosystem. Several factors interfere in the cost of production and consequently in the availability of virus-based formulations. For the *Spodoptera frugiperda multiple nucleopolyhedrovirus* (SfMNPV), employed in the management of the fall armyworm *Spodoptera frugiperda*, factors such as host cannibal habit, temperature, and artificial diet of viral inoculation are important to research. In this work we propose to evaluate a diet based on textured soy protein, without heating during preparation, for viral inoculation and an artificial diet of simplified composition, based on wheat germ, to complete the feeding of larvaes during the stage of viral inoculation in groups of insects. *S. frugiperda* larvae were inoculated with SfMNPV virus in two diets: an artificial diet similar to that of mass rearing of the host and texturized soy protein at the following temperatures and respective ages: at 25 °C at 6 and 8 days after hatching and 31 °C at 4 and 6 days after larvae hatching. And, subsequently, viral inoculation was evaluated in a group of 300 larvae under different incubation times and temperature conditions, complementing feeding at this stage with a diet of simplified composition. The results show that it is possible to inoculate viruses without loss of viral polyhedra production in a textured soy protein-based diet and supplement the larvae diet with an artificial diet of simplified composition to complete the viral infection stage in groups of infected insects.

Keywords: artificial diet, *Baculoviridae*, *Spodoptera frugiperda*, large-scale mass production.

RESUMO
A inoculação de entomopatógenos virais *in vivo* requer estudo de cada patossistema. Diversos fatores interferem no custo de produção e consequentemente na disponibilidade de formulações a base de vírus. Para o vírus *Spodoptera frugiperda multiple nucleopolyhedrovirus* (SfMNPV), empregado no manejo da lagarta do cartucho *Spodoptera frugiperda* (Lepidoptera: Noctuidae), fatores como o hábito canibal do hospedeiro, a temperatura e a dieta artificial de inoculação viral são importantes de serem pesquisados. Nesse trabalho propomos avaliar uma dieta a base de proteína de soja texturizada, sem aquecimento no preparo, para a inoculação viral e uma dieta artificial de composição simplificada, à base de gérmen de trigo, para concluir a alimentação das lagartas durante a etapa de inoculação viral em grupos de insetos. Lagartas de *S. frugiperda* foram inoculadas com o vírus SfMNPV em duas dietas: dieta artificial semelhante à da multiplicação massal do hospedeiro e proteína de soja texturizada nas temperaturas e respectivas idades: à 25 °C com 6 e 8 dias após a eclosão e 31 °C de 4 e 6 dias após a eclosão das lagartas. E, posteriormente, avaliou-se a inoculação viral em grupo de 300 lagartas em diferentes condições

de tempo e temperatura de incubação complementando-se a alimentação nessa fase com dieta de composição simplificada. Os resultados evidenciam que é possível inocular vírus sem perdas de produção de poliedros virais em dieta à base de proteína de soja texturizada, e complementar a alimentação das lagartas com dieta artificial de composição simplificada para concluir a etapa de infecção viral em grupos de insetos infectados.

Palavras-chave: dieta artificial, *Baculoviridae*, *Spodoptera frugiperda*, produção em massa em grande escala.

1 INTRODUCTION

Baculoviruses are a good option for the formulation of biological products, demonstrating efficacy for pest management (GRZYWACZ; MOORE 2017; SOSA-GÓMEZ et al., 2020; VALICENTE et al., 2013). In Brazil, the management of *Anticarsia gemmatalis* in soybean cultivation was what encouraged the use of microbial control with entomopathogenic viruses (MOSCARDI, 1999; MOSCARDI et al., 2011). And after the outbreak with *Helicoverpa armigera* in the country, starting in 2018, some formulations to control *Heliothinae* species, *Spodoptera frugiperda*, and *Chrysodeixis includens* have been registered, among which the virus *Spodoptera frugiperda multiple nucleopolyhedrovirus* (SfMNPV) which is employed in the management of the cartridge larvae *S. frugiperda* (ARDISSON-ARAÚJO et al., 2015; SOSA-GÓMEZ et al., 2020).

The *in vivo* production system is employed for the formulation of SfMNPV based bioinsecticides (VALICENTE; TULHER; BARROS, 2010). The efficiency of insect mass rearing, infrastructure costs, management and artificial or natural diet directly affect the cost of production (ARDISSON-ARAÚJO et al., 2015; SAYED et al., 2021). Therefore the inoculation of viral entomopathogens *in vivo* production systems requires studies focused on the pathogen-host, due to the particularities involved in each pathosystem, such as the food substrate and the temperature of viral inoculation, for example (STINGUEL et al., 2022; SUBRAMANIAN et. al., 2006; ZAMORA-AVILÉS et al., 2017).

To optimize and improve the *in vivo* development technique research conducts trials involving selection studies of viral isolates (VALICENTE; TULHER; BARROS, 2010; VIEIRA et al., 2012), diets for inoculation (ELVIRA et al., 2010a; SAYED et al., 2021; ZAMORA-AVILÉS et al., 2017), age of larvae and optimal temperature for inoculation (SUBRAMANIAN et al., 2006; RIOS-VELASCO et al., 2012), quality of the viral stock (SUBRAMANIAN et al.,

2006; RAMÍREZ-ARIAS et al., 2019), increasing the yield of viral polyhedral (RAMÍREZ-ARIAS et al., 2019), cannibalism and inoculation substrates (ELVIRA et al., 2010a; MACHADO et al., 2021; VALICENTE et al., 2013), substances with hormonal action (ELVIRA et al., 2010a).

In *in vivo* production, the viral inoculation is performed using an artificial diet similar to that used in mass rearing of the host, with a restriction on formaldehyde (VALICENTE; TULHER; BARROS, 2010). But after the viral infection, the larvae remain to feed for another two to three days, and gradually this feeding is reduced. Therefore the use of a complete, nutritionally balanced artificial diet is not necessary since the insect will not complete its development. Thus, the lack of available publications regarding of a diet for viral inoculation that provides viral polyhedral production equivalent to the diet used in mass rearing of the host and provides reduced ingredient costs is amenable the study.

Another issue that is being researched is the homogeneous inoculation of the batch of larvae (ZAMORA-AVILÉS et al., 2017). For this, studies are being developed to add the virus during the preparation of the diet, to make it homogeneous for feeding and consequently ensure greater uniformity in viral infection per batch (ZAMORA-AVILÉS et al., 2017).

Since the standard agar or carrageenan diet requires heating during preparation, it is always necessary to evaluate the temperature at the time of viral inoculation in order not to inactivate the viral polyhedra when adding them to the medium (RIBEIRO; PAVAN, 1994). For the preparation of diets, there are several gelling agents such as alginates, gelatines, gums, glutin, leticin, soy lecithin, CMC (carboxymethyl cellulose), and recently cassava starch (PARRA, 2009; SAYED et al., 2021). The study of gelling agents that do not require heating as a substitute for agar or carrageenan would facilitate the process of virus preparation and mix, in addition to conferring greater homogeneity to the diet when polyhedral inclusion bodies are inoculated only superficially (ZAMORA-AVILÉS et al., 2017).

In addition, proposing an artificial diet option for viral inoculation that contains a simpler composition and whose modifications in composition meet the basic nutritional requirements of the insect during the virus incubation period, period in which there is a reduction in feeding, and promotes viral infection without major contamination and production losses need to be evaluated. The objective of this study is to propose a cold-textured diet based on texturized soy protein for

in vivo inoculation of SfMNPV and a complementary artificial diet of simpler composition, compared to the artificial rearing diet, for virus production in a group of larvae.

2 MATERIALS AND METHODS

The trials were conducted in the Laboratory of Microbial Insect Control, Entomology Sector of NUDEMAFI (Núcleo de Desenvolvimento Científico e Tecnológico em Manejo Fitossanitário de Pragas e Doenças) located in the Centro de Ciências Agrárias e Engenharias of the Universidade Federal do Espírito Santo (CCAE/UFES), in Alegre, Espírito Santo, Brazil.

2.1 MULTIPLICATION OF *SPODOPTERA FRUGIPERDA*

Multiplication and maintenance were carried out in a climate-controlled room at a temperature of 25 ± 2 °C, relative humidity of 60%, and photoperiod of 12 hours. The adults were kept in PVC cages and fed using absorbent cotton soaked in a 10% sucrose solution. For oviposition, white paper sheets were used to cover the inside of the cages and were removed every two days for the removal of the eggs. The white paper with eggs were kept in transparent plastic jars until the neonates hatched. These were transferred with the help of a soft-bristled brush to plastic containers (100 mL) containing an adapted artificial diet based on beans, wheat germ, brewer's yeast, and carrageenan described by Nalim (1991), where they remained for five days. Afterward, approximately 40 larvae were transferred to a gerbox® acrylic container (dimensions 11 x 11 x 3 cm) and after 10 days were individualized in a gerbox® (3 cm diameter) until pupation. When the adults emerged, they were transferred to the rearing cages, continuing the cycle.

2.2 OBTAINING AND PRODUCING *SfMNPV*

The isolate 6 of *Spodoptera frugiperda multiple nucleopolyhedrovirus* (SfMNPV), from the Entomopathogen Bank of the Laboratory of Biological Control of Embrapa Milho e Sorgo, was used for the bioassay because it did not cause liquefaction of the tegument immediately after insect death (VIEIRA et al., 2012). The multiplication of the virus was done from an initial suspension at a concentration of 1 x 10^8 OB/mL, in which pieces of artificial diet (without added formaldehyde) were dipped and offered to the *S. frugiperda* larvae to feed. The insects were kept individualized in plastic containers (50 mL), replenishing the artificial diet whenever necessary.

After infection and death, the cadavers were collected and frozen to be later macerated and purified.

The purification followed the methodology proposed by Hashimoto et al. (2000) with modifications, the infected larvae were macerated in autoclaved distilled water containing 1% SDS (Sodium Dodecyl Sulfate) and filtered in voile tissue to facilitate the removal of the fatty tissue of the larvae and to separate the resulting liquid from the coarser parts of the larvae. The filtrate was left under orbital stirring at 250 rpm for 30 minutes. Afterward, we proceeded with centrifugation at 6.000 rpm/20 minutes, repeating this procedure three times. After the last centrifugation, the precipitate obtained was resuspended in sterile distilled water and stored at 4 °C to be later used in the bioassay.

2.3 DETERMINATION OF OPTIMAL TEMPERATURE AND AGE OF *S. frugiperda* FOR INOCULATION OF *SfMNPV* ON COLD DIET

To select a cold diet for use in the production of the nucleopolyhedrovirus of *S. frugiperda*, preliminary experiments were conducted with the gelling agent at different concentrations of alginate (1, 1.5, 2.0, 2.5, and 3.5%) and calcium chloride (2 and 5%). However, satisfactory results in stiffening the diets were not obtained, or when obtained, feeding off the larvae was inhibited. Thus, it was decided to work with an inoculation diet based on texturized soy protein. A preliminary test was conducted to see if feeding the larvae during the 24h, period corresponding to the inoculation period on texturized soy protein would affect their weight gain compared to the artificial diet. The initial and final weight at 24h of feeding with an artificial diet and texturized soy protein were measured and the difference between the weights was statistically compared. This assay was conducted with larvae reared at 31 °C for 4 days, 31 °C for 6 days, 25 °C for 6 days, and 25 °C for 8 days, with eight repetitions containing 10 insects per repetition. Since there was no interference in weight gain at these ages concerning the use of texturized soy protein for 24 hours, the bioassay was continued, verifying the viral production parameters.

For this, the diets were compared: texturized soy protein (10g/repetition), which was soaked in a virus solution at a concentration of 1 x 10^8 OB/mL for 3 hours before being offered for feeding, and an artificial diet without formaldehyde inoculated with the virus at the same concentration under the surface; and the witness, consisting of the standard diet without virus. In each of these diets (standard diet and texturized soy protein), larvae reared at 25 °C at 6 and 8

days post-hatch and 31 °C at 4 and 6 days. Making 8 treatments with 8 repetitions of 12 insects/repetition, for ease, the treatments were distinguished from T1 to T8 as specified in table (Table 1).

Table 1. Specification of treatments according to the diet for feeding and and larval conditioning of *Spodoptera frugiperda*.

Treatment	Larvae diet and conditioning
T1	Diet artificial 25 °C for 8 days
T2	Diet artificial 25 °C for 6 days
T3	Diet artificial 31 °C for 6 days
T4	Diet artificial 31 °C for 4 days
T5	Texturized soy protein 25 °C for 8 days
T6	Texturized soy protein 25 °C for 6 days
T7	Texturized soy protein 31 °C for 6 days
T8	Texturized soy protein 31 °C for 4 days

Source: Authors

The insects fed on these treatments for 24 hours, then were individualized in an artificial diet and placed in an air-conditioned room at 25 °C. Dead larvae from each treatment were counted, weighed, and stored at 4 °C for further purification and quantification of polyhedral occlusion bodies (OBs). The following were evaluated: (1) Occlusion bodies per larvae (OB/larvae): Total OB/by the number of larvae collected from each repetition; (2) Occlusion bodies per gram (OB/g): total OB/by weight of larvae collected from each repetition; (3) larvae equivalent (LE): $3x10^{11}$/OB/larvae from each repetition, parameter that corresponds to the number of larvae needed to obtain a commercial dose of 3×10^{11} OB to spray one hectare (MAPA 2015); (4) Occlusion bodies by the number of dead larvae per 100 inoculated larvae (OB/100 Larvae): percentage of mortality*OB/larvae. The experimental design was entirely casualization. Data were submitted for analysis of variance at a 5% probability level. For data that did not meet the requirements for normality and homoscedasticity, a non-parametric Kruskal-Wallis test was used at a 5% probability level. When significant, the multiple comparisons test (Dunn) was used. The R statistical program was used to perform the analyses (R Core Team, 2016).

2.4 MEDIUM-SCALE PRODUCTION OF *SFMNPV* IN *S. FRUGIPERDA* LARVAE INOCULATED INTO TEXTURIZED SOY PROTEIN

To analyze the medium-scale production of nucleopoliedrovírus from *S. frugiperda*, we selected the treatment from the previous bioassay that used the artificial diet based on texturized soy protein, and the larvae were multiplied at 31 °C for 6 days, because it resembled most of the

treatments in the parameters evaluated. For this, a plastic container (7cm high, 36.6 cm long, and 26 cm wide) with a lid containing a 5 x 5 cm ventilation opening covered with voile fabric was used as the sampling unit. In each unit, there were 10 grams of texturized soy protein soaked with SfMNPV virus at a concentration of 1 x 10^8 OB/mL for 3 hours before being offered for feeding. We inoculated 300 *S. frugiperda* larvae reared at 31 °C 6 days for 24h on this food source and, after this period, approximately 30 pieces of artificial diet (5g/ pieces), the same used in mass multiplication the larvae, were added and subjected to the following treatments of incubation conditions:

C1: Incubate for 3 days at 31°C, then 3 days at 22°C.

C2: Incubate for 4 days at 31 °C

C3: Incubate for 2 days at 31 °C and then 4 days at 22 °C.

Subsequently, the larvae were frozen for determination and quantification of the viral polyhedra of each treatment. The bioassay was conducted in an entirely casualization design, with five repetitions. The data were submitted to variance analysis at a 5% probability level and the means were compared using Tukey's test.

After selecting the best incubation condition, a complementary bioassay was conducted to compare the provision of the standard artificial diet (NALIM, 1991) and a simpler artificial diet (wheat germ (66.3g/L water), ascorbic acid (4.27g/L water), nipagin (2.64g/L water) and carrageenan (30g/L water) in the production of SfMNPV viral polyhedra. The purpose was to verify the possibility of using a more economical diet in this short period of viral inoculation. The experimental and statistical design followed the same procedure as the previous bioassay.

3 RESULTS

3.1 DETERMINATION OF OPTIMAL TEMPERATURE AND AGE OF *S. frugiperda* FOR INOCULATION OF SFMNPV ON COLD DIET

Utilizing the Kruskall-Wallis test ($p < 0.05$) significant differences were determined for the parameters evaluated among the diets tested (Table 2, 3, 4 and 5).

Table 2. Production parameter OB/100 larvae the baculovirus spodoptera according to diet specification for feeding and larval conditioning (T1 to T8) of *Spodoptera frugiperda*.

	OB/ 100 larvae							
	T1	T2	T3	T4	T5	T6	T7	T8
T2	0.0274	-	-	-	-	-	-	-
T3	0.1754	0.1618	-	-	-	-	-	-
T4	0.0000*	0.0037*	0.0001*	-	-	-	-	-
T5	0.1071	0.2489	0.3787	0.0004*	-	-	-	-
T6	0.0002*	0.0573	0.0052*	0.1354	0.0120*	-	-	-
T7	0.0296	0.4866	0.1702	0.0033*	0.2596	0.0536	-	-
T8	0.0000*	0.0011*	0.0000*	0.3485	0.0001*	0.0681	0.0010*	-
Mean	2.61E+11	1.46E+11	2.31E+11	3.53E+10	1.48E+11	5.96E+10	2.08E+11	3.02E+10
Chi-square	44.97							
Degrees of freedom	7							
p-value	< 0.01							

* P-values differ at 5% probability level by the multiple comparison test (Dunn's test).
Source: Authors

Table 3. Production parameter OB/g the baculovirus spodoptera according to diet specification for feeding and larval conditioning (T1 to T8) of *Spodoptera frugiperda*.

	OB/g							
	T1	T2	T3	T4	T5	T6	T7	T8
T2	0.0698	-	-	-	-	-	-	-
T3	0.3144	0.0250*	-	-	-	-	-	-
T4	0.1011	0.0030*	0.2141	-	-	-	-	-
T5	0.0919	0.0025*	0.1988	0.4786	-	-	-	-
T6	0.3941	0.0404	0.4149	0.1570	0.1444	-	-	-
T7	0.0404	0.0006*	0.1034	0.3192	0.3386	0.0698	-	-
T8	0.4679	0.0813	0.2864	0.0875	0.0793	0.3635	0.0339	-
Mean	1.00E+10	1.98E+10	1.33E+10	7.48E+09	7.36E+09	8.55E+09	9.27E+09	1.14E+10
Chi-square	14.73							
Degrees of freedom	7							
p-value	< 0.04							

* P-values differ at 5% probability level by the multiple comparison test (Dunn's test).
Source: Authors

Table 4. Production parameter Equivalent larvae (LE) the baculovirus spodoptera according to diet specification for feeding and larval conditioning (T1 to T8) of *Spodoptera frugiperda*.

	Lagarta Equivalente LE							
	T1	T2	T3	T4	T5	T6	T7	T8
T2	0.0416	-	-	-	-	-	-	-
T3	0.3444	0.0835	-	-	-	-	-	-
T4	0.0000*	0.0023*	0.0000*	-	-	-	-	-
T5	0.1022	0.3218	0.1826	0.0005*	-	-	-	-
T6	0.0004*	0.0519	0.0011*	0.1144	0.0183*	-	-	-
T7	0.0350	0.4684	0.0717	0.0030*	0.2939	0.0609	-	-
T8	0.0000*	0.0006*	0.0000*	0.3361	0.0001*	0.0519	0.0008*	-
Mean	110.28 ± 11.12	264.47 ± 61.31	140.84 ± 28. 26	786.19 ± 39.54	187.03 ± 17.49	462.25 ± 55.69	238.28 ± 43.22	1128.10 ± 147.75
Chi-square	48.85							
Degrees of freedom	7							
p-value	< 0.01							

* P-values differ at 5% probability level by the multiple comparison test (Dunn's test). Source: Authors

Table 5. Production parameter OB/larvae the baculovirus spodoptera according to diet specification for feeding and larval conditioning (T1 to T8) of *Spodoptera frugiperda*.

	OB/larvae							
	T1	T2	T3	T4	T5	T6	T7	T8
T2	0.0393	-	-	-	-	-	-	-
T3	0.2773	0.1214	-	-	-	-	-	-
T4	0.0000*	0.0020*	0.0000*	-	-	-	-	-
T5	0.0987	0.3192	0.2425	0.0004*	-	-	-	-
T6	0.0003*	0.0493	0.0024*	0.1109	0.0169*	-	-	-
T7	0.0329	0.4679	0.1059	0.0026*	0.2910	0.0581	-	-
T8	0.0000*	0.0005*	0.0000*	0.3337	0.0001*	0.0493	0.0006*	-
Mean	3.02E+09	1.81E+09	3.12E+09	3.89E+08	1.71E+09	7.25E+08	2.46E+09	3.13E+08
Chi-square	48.07							
Degrees of freedom	7							
p-value	< 0.01							

* P-values differ at 5% probability level by the multiple comparison test (Dunn's test).
Source: Authors

The treatment of T2 based on an artificial diet, except for the parameter OB/g which differed from T3, T4, T5, and T7, showed similar results in the other parameters differing only from T4 and T8. The T2 treatment consists of the artificial diet with the virus pipetted superficially, and when analyzing the treatments of virus soaked in texturized soy protein, the one that showed similar to T2 was treatment T7 that evaluated 6-day-old larvae submitted to 31 °C. The treatment T7 was equal to treatments T1, T2, and T3 based on an artificial diet, and T5 and T6 on textured soy protein. The parameter OB/g T7 was equal to all other treatments except T2, and parameters OB/100 larvae, OB/larvae, and larvae equivalent (LE) differ only from T4 and, T8 which refer to larvae subjected to 31 °C for 4 days regardless of inoculation substrate (Table 2, 3, 4, and 5). This result evidences that even though the larvae fed only on texturized protein for 24h did not interfere with viral production. It can be seen that the average OB/larvae was 2.08E+11, very close to the values obtained for the T1 and T2 treatments with on artificial diet used in mass multiplication the host, which was 2.61E+11 and 1.46 E+11, respectively (Table 2).

3.2 MEDIUM-SCALE PRODUCTION OF SfMNPV IN *S. frugiperda* LARVAE INOCULATED INTO TEXTURIZED SOY PROTEIN

The results show that regardless of the incubation condition, polyhedron production was not altered (Table 6). Thus, the incubation condition of 31 ºC for 4 days was chosen, because it has a shorter incubation time (96 hours) and only one temperature (31°C). In the comparison between the wheat germ-based diets and the one used in the mass multiplication the host in the

incubation condition of 31 ºC for 4 days, it can be seen that there was no interference in the weight and production of polyhedra in the diets tested (Table 7). This result is important because besides adding the virus to a cold diet, the feed replacement is also with a more economical diet. In this way, the costs of viral inoculation can be reduced.

Table 6. Medium-scale production parameters viral polyhedra according to incubation conditions (C1, C2 and C3).

	Parameters for medium-scale production of viral polyhedra		
Diet	OB/ container ($x10^{11}$)[1]	OB/g ($x10^{9}$)	g/ container
C1	1.23 ± 0.31 a	6.45 ± 1.33 a	20.06 ± 2.91 b
C2	1.50 ± 0.30 a	4.80 ± 0.58 a	30.12 ± 2.13 a
C3	1.94 ± 0.35 a	7.31 ± 1.26 a	26.30 ± 1.23 ab
CV (%)	45.74	40.17	19.32
p-value	0.310	0.305	0.022

[1]Mean followed by the same letter in the column do not differ at the 5% probability level by Tukey's test.
Source: Authors

Table 7. Parameters of medium-scale production of viral polyhedra in the C2 incubation condition with artificial diet (D2) and wheat germ-based diet (D7).

	Parameters for medium-scale production of viral polyhedra		
Diet	OB/ container ($x10^{10}$)[1]	OB/g ($x10^{9}$)	g/ container
D2	8.60 ± 01.60 a	2.86 ± 0.43 a	29.78 ± 2.42 a
D7	7.18 ± 2.05 a	2.49 ± 0.64 a	29.05 ± 2.54 a
CV (%)	52.13	45.72	18.83
p-value	0.601	0.652	0.84

[1]Mean followed by the same letter in the column do not differ at the 5% probability level by the F test.
Source: Authors

4 DISCUSSION

In Brazil, the highlight for the beginning of the microbial control program with entomopathogenic viruses was the application of *Anticarsia gemmatalis multiple nucleopolyhedrovirus* (AgMNPV) for the management of *Anticarsia gemmatalis* in soybean cultivation (MOSCARDI, 1999; MOSCARDI et al., 2011). Another stimulus was the 2013 outbreak of *Helicoverpa armigera* that obtained good control results provided by imported baculoviruses (ARDISSON-ARAÚJO et al., 2015). In 2018, formulations to control *Heliothinae* species, *Spodoptera frugiperda* and *C. includens* were registered in the country, among which the *Spodoptera frugiperda multiple nucleopolyhedrovirus* (SfMNPV) that is employed in the management of the cartridge larvae *Spodoptera frugiperda* (SOSA-GÓMEZ et al., 2020).

The production of *S. frugiperda* entomopathogenic virus on a commercial scale is *in vivo* and faces several challenges for successful mass multiplication efficiency, mainly the costs of infrastructure, management, and artificial or natural host diet (ARDISSON-ARAÚJO et al.,

2015; SAYED et al., 2021). Therefore the inoculation of viral entomopathogens in *in vivo* production systems requires host-virus studies, due to the particularities involved in each pathosystem, such as the food substrate and the temperature of viral inoculation, for example (STINGUEL et al., 2022; SUBRAMANIAN et al., 2006; ZAMORA-AVILÉS et al., 2017).

The baculovirus, which has the larvae *S. frugiperda* as its host, can be inoculated onto the corn or castor bean leaves, or even into an artificial diet identical to that used in mass rearing except for formaldehyde (MACHADO et al., 2021; STINGUEL et al., 2022; VALICENTE; TULHER; BARROS, 2010). Inoculation in an artificial diet is done after chilling or pipetting superficially and this poses a challenge to ensure a homogeneous infection of the batches of larvae (ZAMORA-AVILÉS et al., 2017; MACHADO et al., 2021).

The problem with mixing the virus incorporated into the artificial diet is the temperature, as it needs to be cooled down to around 50 °C to avoid viral inactivation (CAMACHO et al., 2013; ZAMORA-AVILÉS et al., 2017). And when applied on the surface, some larvae feed on the surface occlusion bodies and others on the lightly contaminated part, and there may be heterogeneity in the infection of the groups of the larvae (ZAMORA-AVILÉS et al., 2017; MACHADO et al., 2021). Studies that discover a cold texture diet become a target for research to solve such problems faced in biofactories.

The result obtained in this study makes it possible to propose changes in the viral inoculation process. The texturized soy protein stood out as a cold texture diet for this purpose, without harming viral infectivity, thermal inactivation, or larval weight gain. The use of texturized soy protein allows you to soak the protein with the virus without worrying about temperature and virus inactivation. In addition, the specific surface area of the protein is much larger than that of the artificial diet, with an ingestion bigger possibility of virus and consequently more homogeneous batches.

One of the goals of the research is to minimize costs during the production process (MOSCARDI et al., 2011; SOSA-GÓMEZ et al., 2020). Currently, the SfMNPV virus is inoculated into an artificial diet identical to that used in mass rearing the host except for formaldehyde (MACHADO et al., 2021; STINGUEL et al., 2022). However, part of this diet is discarded because the larvae die and/or stop feeding due to the viral infection process. Therefore, it becomes even more important to select a low-cost diet that does not interfere with the productivity of occlusion bodies at least in the viral inoculation process step. In this study, the

wheat germ-based diet proved to be an excellent alternative to supplement the diet of infected larvae at this stage of the process, because it does not interfere with virus production and has the advantage of being more economical than the artificial diet used in mass rearing of the host, about the number of ingredients it contains. The study of a low-cost diet that does not interfere in viral productivity has also been researched for the *Spodoptera exigua* larvae virus (ELVIRA et al., 2010b).

Previous studies show that the incubation condition of the virus with lower temperatures reduce cannibalism and contamination, and provide more time for viral replication (SUBRAMANIAN et al., 2006; STINGUEL et al., 2022). But regardless of the condition tested in this study, alternating 31 °C and 22 °C or maintaining 31 °C, there was no statistical difference. Thus, we chose the incubation condition 31 ºC for 4 days (C2) because it has a shorter incubation time (96 hours) and only one temperature. This incubation condition was also analyzed by Machado et al. (2021) who found satisfactory results for SfMNPV production in containers containing different densities of larvae of *S. frugiperda*.

The production of OB per container was 1.50 x 10^{11} in condition C2, and when this was compared to the diet artificial used in mass rearing of the host (D2) and wheat germ-based diet (D7) the production of OB per container was 8.60 x 10^{10} and 7.18 x 10^{10} OB/g, respectively. Zamora-Avilés et al. (2017) inoculating 250 *S. exigua* larvae obtained mean values of 6.0 x 10^{6} and 8.9 x 10^{6} OB/g for agar-based and soy fiber-based diets, respectively. In addition, these authors observed necrophagy and a high level of cannibalism, collecting only about 25 to 35% of the initial number of inoculated larvae. Stinguel et al. (2022) evaluated the cannibalism in *S. frugiperda* larvae inoculated in groups, however, the substrate used was castor bean leaves, and the cannibalism value reached 50% at a density of 50 larvae/recipient e pointing to the need for future studies of OB production by non-cannibalized larvae reared in groups.

Studies proposing viral inoculation in groups of *S. frugiperda* larvae are still scarce in the literature, and in general, the cannibal habit interferes with advances with satisfactory results. Thus, it becomes essential to conduct research that associates substances with the inoculation diet that can minimize cannibalism rates and improve the performance of OB/g productivity. Based on the results obtained in this research in relation to the process of viral inoculation, the use of a cold diet based on textured soy protein and complementing the food with a wheat germ-based diet reduce costs and contribute to adaptations in a mass scale of SfMNPV production.

5 CONCLUSION

Texturized soy protein can be used for the inoculation of *Spodoptera frugiperda multiple nucleopolyhedrovirus* (SfMNPV) *S. frugiperda* larvae inoculated on this food source at 31 °C at the age of 6 days for 24 h showed a good performance in viral production.

The artificial diet of simplified composition based on wheat germ can be used to supplement the feeding after viral infection of the larvaes inoculated in group of 300 individuals. And the optimum incubation condition was 4 days at 31 °C.

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REFERENCES

ARDISSON-ARAÚJO, D.M.P.; SOSA-GÓMEZ, D.R.; MELO, F.L.; BÁO, S.N.; RIBEIRO, B.M. Characterization of *Helicoverpa zea* single nucleopolyhedrovirus isolated in Brazil during the first old world bollworm (Noctuidae: *Helicoverpa armigera*) nationwide outbreak. **Virus Reviews and Research**, v.20, p.1-4, 2015.

CAMACHO, E. J.; GÓMEZ, I. M.; VILLAMIZAR, F. L. Efecto de la temperatura y de dos processos de secado sobre la actividad insecticida de un nucleopoliedrovirus de *Spodoptera frugiperda*. **Revista Mexicana de Ingeniería Química**, v.12, n.3, p. 437-450, 2013.

ELVIRA, S.; GORRIA, N.; MUNOZ, D.; WILLIAMS, T.; CABALLERO, P. A simplified low-cost diet for rearing *Spodoptera exigua* (Lepidoptera: Noctuidae) and its effect on *S. exigua* nucleopolyhedrovirus production. **Journal of Economic Entomology**, v.103, n.1, p.17-24, 2010.

ELVIRA, S.; WILLIAMS, T.; CABALLERO, P. Juvenile hormone analog technology: effects on larval cannibalism and the production of *Spodoptera exigua* (Lepidoptera: Noctuidae) nucleopolyhedrovirus. **Journal of economic entomology**, v.103, n.3, p. 577-582, 2010.

GRZYWACZ, D., MOORE, S., 2017. Production, formulation, and bioassay of baculoviruses for pest control. In: Lacey, L.A. (Ed.), **Microbial Control of Insect and Mite Pests.From Theory to Pratice**. Academic Press, San Diego, CA, p. 109–124.

HASHIMOTO, Y.; HAYASHI, K.; HAYAKAWA, T.; UENO, Y.; SHIMOJO, E.I.; KONDO, A.; MIYASONO, M.; SANO, Y.; MATSUMOTO, T.; GRANADOS, R.R. Physical map of a *Plutella xylostella* granulovirus genome. **Applied Entomology and Zoology**, v.35, n.1, p.45-51, 2000.

MACHADO, L.C.; SANTOS JUNIOR, H. J. G. DOS; MAURI, L.V.R.; GUILHEN, J.H.S.; PAIVA, C.E.C. Aperfeiçoamento da Técnica de Produção Massal *in vivo* de Baculovírus spodoptera. **Revista em Agronegócio e Meio Ambiente**, v.14, n.3, p.1-13, 2021.

MOSCARDI, F. Assessment of the application of baculoviruses for control of Lepidoptera. **Annual Review of Entomology**, v.44, p.257-289, 1999.

MOSCARDI, F.; SOUZA, M.L.; CASTRO, M.E.B.; MOSCARDI, M.L.; SZEWCZYK, B. (2011). Baculovirus Pesticides: Present State and Future Perspectives. In: I., Ahmad, F., Ahmad, & J., Pichtel (Eds.), **Microbes and Microbial Technology: Agricultural and Environmental Applications**. Nova York: Springer, p.415-445, 2011.

NALIM, D. M. (1991). Biologia, nutrição quantitativa e controle de qualidade de populações de Spodoptera frugiperda (J. E. Smith, 1797) (Lepidoptera: Noctuidae) em duas dietas artificiais. Universidade de São Paulo Biblioteca digital da USP.

PARRA, J.R.P. (2009). A evolução das dietas artificiais e suas interações em ciência e tecnologia. In: A.R., PANIZZI; J.R.P., PARRA (Eds.), **Bioecologia e nutrição de insetos Base para o manejo integrado de pragas**. Brasília, DF: Embrapa Informação Tecnológica, p.91-174.

RAMÍREZ-ARIAS, F.G.; LASA, R.; MURILLO, R.; NAVARRO-DE-LA-FUENTE, L.; GABRIEL MERCADO, G.; WILLIAMS, T. Post-mortem incubation influences occlusion body production in nucleopolyhedrovirus-infected larvae of *Spodoptera frugiperda*. **Biological Control**, v.135, p. 33-40, 2019.

RIBEIRO, H.C.T.; PAVAN, O.H. Baculovirus thermal stability. **Journal of thermal biology**, v.19, n.1, p.21-24, 1994.

RIOS-VELASCO, C.; GALLEGOS-MORALES, G.; BERLANGA-REYES, D., CAMBERO-CAMPOS, J.; ROMO-CHACÓN, A. Mortality and production of occlusion bodies in *Spodoptera frugiperda* larvae (Lepidoptera: Noctuidae) treated with nucleopolyhedrovirus. **Florida Entomologist**, v.95, n.3, p.752-757, 2012.

SAYED, W.A.A.; EL-HELALY, A.; JAMAL, Z. A.; EL-BENDARY, H. Effect of a low cost diet on the cotton leaf worm, *Spodoptera littoralis* nucleopolyhedrosis virus pathogenicity and sterile insect technique. **Egyptian Journal of Biological Pest Control**, v.31, n.117, p.1-8, 2021.

SOSA-GÓMEZ, D.R.; MORGADO, F.S.; CORRÊA, R.F.T.; SILVA, L.A., ARDISSON-ARAÚJO, D.M.P.; RODRIGUES, B.M.P.; OLIVEIRA, E.E., AGUIAR, R.W.S.; RIBEIRO, B.M. Entomopathogenic Viruses in the Neotropics: Current Status and Recently Discovered Species. **Neotropical Entomology**, v.49, p.315–331, 2020.

STINGUEL, P.; PAIVA, C.E.C.; ZUIM, V.; AZEVEDO, A.C.T.; VALICENTE, F.H.; DOS SANTOS JÚNIOR, H.J.G. Optimization of In Vivo Production of *Spodoptera frugiperda* multiple nucleopolyhedrovirus (SfMNPV). **Neotropical Entomology**, v.51, n.1, p.122-132, 2022.

SUBRAMANIAN, S.; SANTHARAM, G.; SATHIAH, N.; KENNEDY, J.S.; RABINDRA, R.J. Influence of incubation temperature on productivity and quality of *Spodoptera litura nucleopolyhedrovirus*. **Biological Control**, v.37, n.3, p. 367-374, 2006.

VALICENTE, F.H.; TUELHER, E.S.; PENA, R.C.; ANDREAZZA, R.; GUIMARÃES, M.R.F. Cannibalism and virus production in *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) larvae fed with two leaf substrates inoculated with Baculovirus spodoptera. **Neotropical Entomology**, v. 42, n. 2, p. 191-199, 2013.

VALICENTE, F.H.; TULHER, E.S.; BARROS, E.C. **Processo de produção comercial de baculovírus em grande escala**. Sete Lagoas: EMBRAPA-CNPMS, 2010. 6p. (Circular Técnica, 157).

VIEIRA, C.M.; TUELHER, E.S.; VALICENTE, F.H.; WOLFF, J.L.C. Characterization of a *Spodoptera frugiperda multiple nucleopolyhedrovirus* isolate that does not liquefy the integument of infected larvae. **Journal of Invertebrate Pathology**, v.111, n,2, p.189-192, 2012.

ZAMORA-AVILÉS, N.; MARTÍNEZ, A.M.; PINEDA, S.; BRAVO-PATIÑO, A.; FIGUEROA, I.; LASA, R. Cool-textured diets for use in baculovirus production. **Biocontrol Science and Technology**, v.27, n.11, p. 1327-1338, 2017.