Research

Biological activity of two Mexican nucleopolyhedrovirus isolates and sublethal infection effects on *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae)

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Abstract

The biological activity of 2 isolates of Spodoptera frugiperda multiple nucleopolyhedrovirus (Sf-YUC and Sf-CHI) obtained from the states of Yucatán and Chiapas, Mexico, on second instar fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) from Michoacán State, Mexico, was determined and compared with that of a Nicaraguan isolate (Sf-NIC). Response of third and fourth instar *S. frugiperda* to the most active isolate, Sf-YUC, also was determined. Sublethal effects caused by this isolate and its intergenerational persistence were evaluated. The most pathogenic isolates on second instar *S. frugiperda* were Sf-NIC and Sf-YUC. No significant differences were detected in the speed of kill between the Sf-NIC (146 h) and Sf-YUC (149 h) isolates, whereas that of the Sf-CHI (158 h) isolate was slower significantly. The lethal concentration that kills 50% of the insects (LC_{50}) values of the Sf-YUC isolate increased with larval stage from 9.45×10^4 to 1.25×10^6 occlusion bodies per mL. Statistically significant reductions in pupal weight, fecundity, fertility, and adult longevity were associated in individuals derived from third instar (generation F_0) treated with 4.8×10^4 occlusion bodies per mL of the Sf-YUC isolate. A viral mortality of 15.83 ± 1.43% in larvae as well as a significant reduction in pupal weight of generation F_1 was recorded. In conclusion, the Mexican isolates may prove suitable as the basis for biological insecticides for regional control of *S. frugiperda*. Sublethal infections that persist between generations could incur developmental costs and decrease reproductive capacity of the host insect.

Keys Words: baculoviruses; virus persistence; sublethal effects; fall armyworm

Resumen

En el presente estudio, se determinó la actividad biológica de dos aislados mexicanos del nucleopolyhedrovirus múltiple de Spodoptera frugiperda (Sf-YUC y Sf-CHI) sobre larvas de segundo instar del gusano cogollero, *Spodptera frugiperda* (J. E. Smith), y se compararon con un aislado nicaragüense (Sf-NIC). También se determinó la respuesta de tercer y cuarto instar de *S. frugiperda* al aislado mexicano más activo, Sf-YUC. Finalmente, se evaluaron los efectos subletales causados por este aislado y su persistencia intrageneracional. Los aislados más patogénicos sobre el segundo instar de *S. frugiperda* fueron Sf-NIC y Sf-YUC. No se detectaron diferencias significativas en la velocidad de muerte entre los aislados Sf-NIC (146 h) y Sf-YUC (149 h), mientras que la del aislado Sf-CHI (158 h) fue significativamente mayor. Los valores de la concentración letal que matan el 50% de los insectos (CL_{50}) se incrementaron con el estado larval desde 9.45×10^4 a 1.25×10^6 cuerpos de oclusión por mL del segundo al cuarto instar. Reducciones estadísticamente significativas en el peso pupal, la fecundidad, la fertilidad y la longevidad de adultos se asociaron con individuos derivados de tercer estadio (generación F_0) tratados con $4{,}8 \times 10^4$ cuerpos de oclusión por mL del aislado Sf-YUC. observó una reducción significativa en el peso pupal. En conclusión, los aislados mexicanos pueden ser adecuados como la base de insecticidas biológicos para el control de *S. frugiperda*. Las infecciones subletales que persisten entre generaciones pueden generar costos de desarrollo y disminuir la capacidad reproductiva del insecto huésped.

Palabras Clave: baculovirus; persistencia de viral; efectos subletales; gusano cogollero

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is the most serious maize pest in the Americas (Nagoshi & Meagher 2004; Blanco et al. 2014). Since 2016 it has spread rapidly through the temperate areas of other continents and successfully established in Africa, China, and Oceania (Goergen et al. 2016; CABI 2020; Jing et al. 2020). Chemical control, through the use of organosynthetic insecticides (i.e., organophosphates, carbamates, and pyrethroids), has been the most common method of reducing *S.*

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frugiperda populations; however, their excessive use has negative impact on the health of farmers, and populations of beneficial organisms (parasitoids and predators) (Tinoco & Halperin 1998; Williams et al. 1999; Blanco et al. 2014), and may favor the development of resistance to these compounds (Wyckhuys et al. 2013).

A promising alternative for management of *S. frugiperda* is integration of common control practices with management through natural enemies, including microbial agents (Ríos-Velasco et al. 2011; García-Gutiérrez et al. 2013). The Spodoptera frugiperda multiple nucleopolyhedrovirus is a baculovirus that has been isolated from fall armyworm populations in the US, Nicaragua, Colombia, Argentina, Mexico (Berretta et al. 1998; Escribano et al. 1999; Barrera et al. 2011; Ríos-Velasco et al. 2011; García-Banderas et al. 2020; Hussain et al. 2021), and recently in China (Lei et al. 2020). Due its insecticidal activity, in the field this baculovirus can cause *S. frugiperda* mortalities of about 80% (Valicente & Da Costa 1995; García-Banderas et al. 2020). However, biological activity of different genotypes of the same baculovirus is variable (Serrano et al. 2015). In this regard, the variability in the biological activity of nucleopolyhedrovirus isolates could be related to both genetic changes and host response. Genetic changes are generated through various mechanisms, including point mutation, insertion and deletion, or recombination between different genotypes while coinfecting a single cell (Ikeda et al. 2015). The phenotypic variation of the viruses also may depend on the resource quality and interactions that alter the host immune system (Cory & Franklin 2012), including antiviral activity, phagocytosis, and apoptosis responses (Briese 1986; Ikeda et al. 2015).

Recently, our research group reported that 2 native Mexican SeMNPV isolates (Sf-YUC and Sf-CHI) may prove suitable as the basis for biological insecticides to control *S. frugiperda* (García-Banderas et al. 2020). However, it is necessary to expand knowledge of the activity of these nucleopolyhedroviruses, including dose-response characteristics at different host ages (Milks 1998), persistence in the host, and sublethal effects (Rothman & Myers 2000).

Generally, *S. frugiperda* can be found in all developmental stages of maize and all cropping seasons because of overlapping generations (Villa-Castoñera & Catalán-Valencia 2004), making data on stage-related virulence of practical importance. In addition, sublethal nucleopolyhedrovirus infections also increase possible opportunities for vertical transmission of infection from parents to offspring, which may be an important mechanism of virus survival (Rothman & Myers 2000; Cabodevilla et al. 2011). Although response of several *S. frugiperda* instars to Spodoptera frugiperda multiple nucleopolyhedrovirus (Escribano et al. 1999; Ríos-Velasco et al. 2011) and its sublethal effects (Martínez et al. 2005) have been reported, the magnitude of differences in susceptibility to virus or sublethal infection can vary in each baculovirus-host system.

The objectives of this work were to determine (i) the biological activity of 2 recently identified Mexican Spodoptera frugiperda multiple nucleopolyhedrovirus isolates (Sf-YUC and Sf-CHI) on second instar *S. frugiperda*, (ii) the response of third and fourth instars of this insect to the most active isolate, (iii) sublethal effects in terms of pupal weight and adult reproduction, and (iv) the transgenerational persistence of the Sf-YUC isolate.

Materials and Methods

INSECTS AND REARING

The insects used in this study were from 2 colonies: *S. frugiperda*-laboratory (Sf-lab) and *S. frugiperda*-Tarímbaro (Sf-Tarímbaro). The Sf-lab colony had been maintained for 1 yr in the laboratory of the Instituto de Investigaciones Agropecuarias y Forestales, Universidad Michoacana de San Nicolás de Hidalgo (El Trébol, Michoacán, Mexico). The Sf-Tarímbaro colony was collected from a maize field in Tarímbaro, within a 21 km radius from the city of Morelia, Michoacán, Mexico. Larvae of both colonies were reared on an artificial diet (Poitout & Bues 1974), and the adults were fed a 15% honey solution. The colonies were maintained in a controlled environment chamber at 25 ± 2 °C and 65 ± 5% RH, with a photoperiod of 16:8 h (L:D).

The Sf-lab colony was used for virus amplification and identification, whereas the Sf-Tarímbaro colony was used to determine biological activity, sublethal effects, and transgenerational persistence (as described below).

VIRUS ISOLATES AND AMPLIFICATION

The 2 Mexican Spodoptera frugiperda multiple nucleopolyhedrovirus isolates used in this work, Sf-YUC and Sf-CHI, recovered from the soil of maize plots in Yucatán and Chiapas, Mexico, respectively, were provided by Trevor Williams (Instituto de Ecología, Xalapa, Veracruz, Mexico). The Spodoptera frugiperda multiple nucleopolyhedrovirus isolate has not been commercialized in Mexico and, to our knowledge, there is no history of applications of this virus to control *S. frugiperda* in any of the collection sites of these isolates. As a reference standard, we used a virus isolate from Nicaragua (Sf-NIC), which was previously characterized by Escribano et al. (1999).

The Mexican viruses were confirmed as Spodoptera frugiperda multiple nucleopolyhedrovirus isolates using the polymerase chain reaction (PCR) method. Viral DNA was obtained from a purified suspension of each isolate at a concentration of 1×10^9 occlusion bodies per mL. An aliquot (300 µL) of each viral suspension was mixed with 500 µL extraction buffer (100 mM tris HCl, 50 mM EDTA, and 1.4 M NaCl, 50 µL CTAB 10% [Hexadecyltrimethylammonium bromide], 50 µL SDS 20%) and 5 µL Proteinase k). Viral genomic DNA was extracted with chloroform-isoamyl alcohol precipitated by adding 640 µL isopropanol, and 60 µL sodium acetate (3.5 M) was then added. The DNA pellet was re-suspended in 50 µL of milli-Q water. Specific oligonucleotides used for Spodoptera frugiperda multiple nucleopolyhedrovirus were designed based on the studies of De Moraes and Maruniak (1997), with an expected fragment size of 575 bp. The DNA and PCR products were observed in 2% TAE agarose gel stained with 0.5 µg per mL ethidium bromide and photo-documented in a UV transilluminator (BioDoc-it^2® Imager, Upland, California, USA).

Each viral isolate was amplified by feeding early fourth instar *S. frugiperda* with a semisynthetic diet contaminated by the virus on the surface. The larvae were observed daily until death; occlusion bodies from the cadavers were then purified as described by Muñoz et al. (1997) with some modifications. The occlusion body pellet was re-suspended in distilled water, and virus concentration was determined using an improved Neubauer haemocytometer (Hawskley, Lancing, United Kingdom) under phase contrast microscopy (Olympus BX41; Guadalajara, Jalisco, Mexico) at 400× magnification. Purified occlusion bodies were stored at -20 °C.

BIOLOGICAL ACTIVITY

To determine the biological activity of the Sf-NIC, Sf-YUC, and Sf-CHI isolates on *S. frugiperda* larvae, 3 bioassays were performed. In the first bioassay, newly molted (12-h-old) second instar *S. frugiperda* were inoculated with 5 different concentrations (1.9×10^3, 9.6×10^3, 4.8×10^4, 2.4×10^5, and 1.2×10^6 occlusion bodies per mL) of these 3 isolates using the droplet-feeding method (Hughes & Wood 1981). The occlusion bodies were re-suspended in an aqueous suspension con-

taining 20% sucrose, and 0.01% (v/v) blue artificial dye (McCormick®, Inc., Hunt Valley, Maryland, USA). Larvae were starved for 12 h before the bioassay to induce a higher feeding rate. Larvae that ingested the drop within a 10 min period were individually transferred to 24-well Costar® tissue culture plates (Corning®, New York, New York, USA) and provided with a virus-free semi-synthetic diet. The bioassays were kept in a controlled environment chamber at 25 ± 2 °C and 65 ± 5% RH at a 16:8 h (L:D) photoperiod. Four replicates of 24 individuals were performed for each concentration and isolate. Similarly, 4 replicates of 24 larvae treated with only distilled water plus blue artificial coloring and the sucrose solution were used as a control for each isolate. Larval mortality was recorded every 24 h starting on the third d post-inoculation, when the first larvae killed by viruses were observed. The bioassay was observed for 11 d.

In the second bioassay, mean time to death was determined on second instar *S. frugiperda.* This bioassay was performed using the same procedure described previously, but in this case the concentrations of Sf-NIC, Sf-YUC, and Sf-CHI isolates were estimated to result in about 90% mortality (4.8×10^5, 1.5×10^7, and 3.1×10^7 occlusion bodies per mL, respectively), and larval mortality was checked at 12 h intervals until 192 h. Bioassays using 24 larvae per isolate and control were replicated 4 times.

The third bioassay was performed using the procedure described for the first bioassay, but in this case biological activity was determined for newly molted (12-h-old) third and fourth instar *S. frugiperda* using the Sf-YUC isolate only. Both third and fourth instar larvae were starved for 12 h before the bioassay to induce a higher feeding rate. Five different concentrations were fed to larvae of each instar: for third instar, 9.6×10^3, 4.8×10^4, 2.4×10^5, 1.2×10^6, and 6.0×10^6 occlusion bodies per mL; and for fourth instar, 9.7×10^3, 7.8×10^4, 6.2×10^5, 5.0×10^6, and 4×10^7 occlusion bodies per mL.

SUBLETHAL EFFECTS

One hundred third instar (12-h-old) *S. frugiperda* were inoculated using the droplet-feeding method mentioned above with a concentration (4.8×10^4 occlusion bodies per mL) estimated to cause about 40% mortality by the Sf-YUC isolate. One hundred third instars, treated only with distilled water containing 20% sucrose and 0.01% blue artificial dye, were used as control. Larvae were weighed prior to inoculation. After inoculation, larvae were individualized in 29 mL plastic pots (SOLO® Cup Company, Chicago, Illinois, USA) containing semi-synthetic virus-free diet. The experiment was performed 3 times. The larvae were checked at 12 h intervals, starting on the third d after virus inoculation, for pupation or until mortality occurred. The bioassay was carried out under the same environmental conditions, as detailed in the section above. Sublethal effects were recorded in terms of pupal weight and adult reproduction (fecundity and fertility) in individuals derived from third instar larvae, treated and non-treated (control).

To determine pupal weight, 181 pupae from treated larvae and 133 non-treated pupae were individually weighed 4 d after pupation. Pupal sex ratio was assessed based on examination of the seventh, eighth, and ninth sternoabdominal segments (Sannino et al. 1987) using a stereoscopic microscope (400×) (Carl Zeiss Stemi DV4, Berlin, Germany). Pupae were then placed by sex in 0.5 L plastic containers (Reyma®, León, Guanajuato, Mexico), and after 9 d were examined daily for adult emergence.

After adult emergence, 10 pairs (generation F_0; < 12-h-old) were selected randomly from virus and control treatments. Each pair was placed separately in an oviposition brown paper bag (10.8 × 23.5 cm) (Grupo Surtidor®, Morelia, Mexico) and provided with a 15% honey solution administered with moist cotton. The bag was replaced every 24 h and fecundity was determined by counting the total number of eggs laid by each female until death. The percentage of eggs that hatched from those collected from the third batch of oviposition was used to evaluate fertility. The number of eggs that hatched was assessed 4 d after collection when egg hatching was complete in the control group. Duration of the egg stage also was determined. Longevity of the adults of each treatment was determined by observation every 24 h until their death. All experiments were performed 3 times.

TRANSGENERATIONAL PERSISTENCE

To observe the transgenerational persistence of the Sf-YUC isolate, 560 and 510 newly hatched *S. frugiperda* larvae (generation F_1) were obtained from the third oviposition batch from generation F_0 adults of both virus and control treatments, respectively. These larvae were placed individually into cylindrical wells (1.6 cm bottom diam) of a 24-well Costar® tissue culture plates (Corning®, New York, New York, USA) containing semi-synthetic virus-free diet. The larvae were checked at 24 h intervals until pupation. Larval mortality by virus was recorded. The weight and sex ratio of pupae also were recorded.

STATISTICAL ANALYSIS

In the biological activity assay, mortality data were subjected to probit regression using the POLO-PC program (LeOra Software 1987). An improved fit to the probit model was observed at 192 h post-inoculation for second and third instar *S. frugiperda* and at 216 h post-inoculation for fourth instar. Potency ratio was calculated as the ratio of the effective concentration relative to that of the exotic isolate (Sf-NIC) to compare isolates and insect age. If the 95% confidence interval of this ratio included 1.0, then the LC_{50} values were considered to be not significantly different (Robertson et al. 2017).

The values for mean time to death were estimated using mortality data, and the analysis was performed using the Generalized Linear Interactive Modelling (GLIM) program with a specified Weibull distribution (Crawley 1993). Effects on pupal weight, reproduction (fecundity and fertility), and egg stage duration of the virus and control treatments were analyzed by the *t*-student test. The initial weight values of the third instar *S. frugiperda* before inoculation were log-transformed for analysis by the *t*-student test. Mean values were tested for normality and homoscedasticity of variance (Levene's test) prior to analysis. Adult longevity of generation F_0 and pupal weight of generation F_1 were analyzed by the non-parametric Mann-Whitney U test. Sex ratio of generations F_0 and F_1 pupae was analyzed with contingency tables (χ^2 test). These data were processed using the IBM SPSS version 21 statistical package (IBM Corp., Armonk, New York, USA).

Results

BIOLOGICAL ACTIVITY

The Sf-NIC and Sf-YUC isolates were pathogenic similarly to second instar *S. frugiperda*, and the Sf-CHI isolate was 11- and 3-fold less pathogenic, respectively (Table 1). The Sf-NIC (146 h, fiducial limits 140–152) and Sf-YUC (149 h, fiducial limits 140–156) isolates were virulent similarly, whereas the Sf-CHI isolate was 1.1-fold less virulent (158 h, fiducial limits 149–169) (Weibull survival analysis, $P > 0.05$). Mean time to death values were estimated for virus concentrations that resulted in 80 to 97% larval mortality. *Spodoptera frugiperda* larvae were less susceptible to Sf-NIC isolate infection as their developmental stage advanced. Relative potencies and 95% lethal concentration for second instar with respect to third and fourth instar showed a statistically significant decrease in susceptibility (10- and 13-fold, respectively).

Table 1. LC_{50} values and relative potencies of 3 SfMNPV isolates for second instar of *Spodoptera frugiperda* and LC_{50} values of Sf-YUC isolate for second, third, and fourth instars *S. frugiperda.*

Isolates per instar	LC_{50} (occlusion bodies per mL)	Confidence limits 95%	df	Intercept ± SE	χ^{2b}	RP[c]	Confidence limits 95%
Sf-NIC[a]	2.85×10^4	$1.44 \times 10^4 – 5.23 \times 10^4$	3	−2.29 ± 0.34	1.1	1	—
Sf-YUC[a]	9.45×10^4	$4.31 \times 10^4 – 2.34 \times 10^5$	3	−2.42 ± 0.26	1.1	0.3	0.11 – 0.84
Sf-CHI[a]	2.99×10^5	$7.46 \times 10^4 – 9.62 \times 10^6$	3	−2.82 ± 0.26	4.9	0.09	0.03 – 0.27
Sf-YUC							
Second	9.45×10^4	$4.31 \times 10^4 – 2.34 \times 10^5$	3	−2.42 ± 0.26	1.1	1	—
Third	9.44×10^5	$4.70 \times 10^4 – 2.11 \times 10^6$	4	−3.81 ± 0.34	4.6	0.1	0.041 – 0.62
Fourth	1.25×10^6	$3.44 \times 10^5 – 3.90 \times 10^6$	3	−3.34 ± 0.43	5.4	0.076	0.026 – 0.22

Probit regressions were fitted using the PoloPlus program.
[a]Bioassays performed on second instar *S. frugiperda*; [b]Goodness-of-fit chi-square; [c]Relative potencies (RP) were calculated as the ratio of effective concentrations relative to Sf-NIC isolate on 2nd instar

SUBLETHAL EFFECTS

Initial weight of third instar *S. frugiperda* larvae (1.63 ± 0.02, *n* = 294) before inoculation with 4.8×10^4 occlusion bodies per mL of the Sf-YUC isolate was not significantly different from the control (1.66 ± 0.01, *n* = 261) (*t* = −0.27; df = 580; *P* = 0.79). Eleven d after inoculation with this isolate, a mean percentage of virus mortality of 42.2 ± 3.9% was obtained (ranging from 37.8 to 50%), which was expected of the concentration used. The Sf-YUC isolate did not affect either weight or proportion of female pupae relative to the control individuals and those that survived virus infection (Table 2). In both virus and control treatments, adult emergence was 100%.

Both the cumulative number of eggs laid per female during its lifetime and percentage of egg hatch in the virus treatment were significantly lower (1.2-fold) than in the control (Table 2). No significant differences in duration of the egg stage were observed between the control and virus treatment (Table 2). The adults emerging from larvae that survived virus infection had significantly shorter longevity than those of the control (8.16 ± 1.53 vs. 10.73 ± 0.98 d) (Mann Whitney *U* = 63.0; df = 1; *P* < 0.001).

TRANSGENERATIONAL PERSISTENCE

Viral mortality was low in generation F_1 larvae of the virus treatment (15.83 ± 1.43%, *n* = 554). No viral mortality was observed in the control. Pupal weight was significantly lower in the virus treatment (154.84 ± 1.13, *n* = 436) than in the control (166.86 ± 1.04 mg, *n* = 462) (Mann-Whitney *U* = 6.32; df = 1; *P* < 0.0001). No significant differences in the proportion of female pupae were observed between the virus treatments (54.82%, *n* = 267) and the control (51.51%, *n* = 255) (χ^2 = 1.1; df = 1; *P* = 0.30).

Discussion

Laboratory and field studies have determined that isolates of Spodoptera frugiperda multiple nucleopolyhedrovirus have potential insecticidal use in biological control of *S. frugiperda* (Ríos-Velasco et al. 2011; García-Gutiérrez et al. 2013; García-Banderas et al. 2020). In general, some of the LC_{50} values obtained in our study, including the reference isolate (Sf-NIC) (range from 2.85×10^4 to 2.99×10^5 occlusion bodies per mL), are in agreement with previous studies using the same Spodoptera frugiperda multiple nucleopolyhedrovirus isolates and similar experimental conditions (range from 6.1×10^3 to 1.25×10^5 occlusion bodies per mL) (García-Banderas et al. 2020).

However, these authors also observed that the pathogenicity and virulence of these viruses on second instar *S. frugiperda* differed among Mexican *S. frugiperda* populations. For example, in an *S. frugiperda* population collected in southern Mexico (Chiapas State), both Sf-YUC and Sf-CHI isolates were less pathogenic than the Sf-NIC isolate, but the speed of kill of Sf-YUC was fastest. In contrast, in larvae collected from another Mexican region (Sinaloa State), similar levels of activity and virulence were observed between the Sf-NIC and Sf-YUC isolates. In addition, in the García-Banderas et al. (2020) study, in larvae from a population collected in Michoacán State, in a geographically different site from our population, Sf-YUC was more active than Sf-NIC and the former isolate was the most virulent. However, in our study both Sf-YUC and Sf-NIC isolates were more pathogenic than the Sf-CHI isolate, but similar levels of speed of kill were observed among all isolates.

Differences or similarities in host response to different nucleopolyhedrovirus isolates also have been observed in the same host- Spodoptera frugiperda multiple nucleopolyhedrovirus system studied here (Escribano et al. 1999; Ríos-Velasco et al. 2011) or other host-baculovirus systems (Cory et al. 1997; Cabodevilla et al. 2011; Serrano et al. 2015; Zamora-Avilés et al. 2017). This could indicate that local adaptation in the host-pathogen system may depend on ecological, epidemiological, or genetic factors (Adiba et al. 2010). For this reason, the selection of native isolates that are suitable for development as biological control agents requires the characterization of the isolate present in each geographical region (Figueiredo et al. 2009).

Table 2. Effects (mean ± SE) of Sf-YUC isolate on development and reproduction biological parameters when third instar *Spodoptera frugiperda* larvae were treated by ingestion with 4.8×10^4 occlusion bodies per mL.

	Treatments	
Biological parameters	Control (*n*)	Virus (*n*)
Pupal weight[a]	150.5 ± 2.06 a (181)	151.9 ± 2.48 a (133)
Sex ratio (% females)[b]	53.78 a (71)	42.10 a (56)
Mean of eggs per female[c]	1561.87 ± 0.78 a (30)	1294.0 ± 27.48 b (29)
Percent egg hatch[d]	91.63 ± 1.10 a	73.0 ± 3.73 b
Egg stage duration (d)[e]	4.4 ± 0.10 a	4.50 ± 0.09 a

Means ± SE within the same row followed by the same letter are not significantly different.
[a]*t*-test = −0.45; df = 312; *P* = 0.65; [b]χ^2 = 0.38; df = 1; *P* = 0.54; [c]*t*-test = 6.48; df = 57; *P* < 0.001; [d]*t*-test = 5.35; df = 57; *P* < 0.001; [e]*t*-test = −0.719; df = 58; *P* = 0.47

Several studies have reported a decrease in susceptibility to virus infection among older larvae (Escribano et al. 1999; Ríos-Velasco et al. 2011). Similarly, in our study, the Sf-YUC isolate against second to fourth instars demonstrated that LC_{50} values increased with larval stage. The incidence of *S. frugiperda* larvae can show different distribution patterns in maize fields (Melo et al. 2006) and different larval instars commonly are present during all growing seasons of this crop (García-Banderas et al. 2020). For this reason, the relationship age-dose is essential for the development of predictive control methods (Payne et al. 1981).

Debilitating effects such as reduction in fecundity and fertility, alteration of development time, and reduction in body weight have been observed in the survivors of baculovirus infection (Myers et al. 2000; Matthews et al. 2002). However, the frequency of sublethal infections can vary in each virus system and depend on the larval stage at inoculation and the occlusion body dose ingested (Cabodevilla et al. 2011).

In the present study, no significant differences were observed in pupal weight and sex ratio of surviving insects that were inoculated in the third instar compared with non-inoculated insects. Similarly, no significant effects were observed on either pupal weight or sex ratio of surviving insects inoculated with Spodoptera frugiperda multiple nucleopolyhedrovirus in second and fifth instar *S. frugiperda* (Martínez et al. 2005). However, despite low viral mortality (16%) observed in generation F_1 larvae, pupal weight in the virus treatment was 1.1-fold lower than in the control. This may show that debilitating effects of persisting Spodoptera frugiperda multiple nucleopolyhedrovirus on the host may be associated with biological cost to the insect host to combat the disease (Williams et al. 2017).

Covert infection with a Spodoptera frugiperda multiple nucleopolyhedrovirus isolate in *S. frugiperda* adults was confirmed by reverse transcription PCR of virus gene transcripts (*polh*) (Martínez et al. 2005). Similarly, molecular techniques have demonstrated intra- or intergenerational persistence of the other nucleopolyhedroviruses (e.g., BmNPV and SeMNPV) that incur costs to developmental and reproductive capacity of the host insect (Khurad et al. 2004; Cabodevilla et al. 2011). We observed that reproduction of *S. frugiperda* adults from larvae inoculated with the virus was significantly affected. This coincided with previous studies, where a significant reduction in the fecundity (about 46%) caused by sublethal infections in larvae of this insect by a Spodoptera frugiperda multiple nucleopolyhedrovirus isolate also was observed (Martínez et al. 2004). Reductions in fecundity may be due to pathological damage to infected reproductive tissues, as occurred in *Malacosoma californicum pluviale* (Dyar) (Lepidoptera: Lasiocampidae) (Rothman & Myers 1994) and *Bombyx mori* L. (Lepidoptera: Bombycidae) (Khurad et al. 2004) females emerged from larvae infected with their homologous nucleopolyhedroviruses.

We confirmed that the Sf-YUC isolate is a promising bioinsecticide for control of *S. frugiperda*. The impact of Sf-YUC on the surviving host, in terms of development and reproduction, could affect future generations and thereby favor the control of *S. frugiperda*. Further studies are needed to determine genotypic diversity of the Mexican isolates and evaluate the contribution of possible genotypic variants to insecticidal properties.

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