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Effect of four multiple nucleopolyhedrovirus isolates on the larval mortality and development of *Spodoptera exigua* (Lepidoptera: Noctuidae): determination of virus production and mean time to death

Juan Carlos Luna-Espino^{1,2}, Víctor R. Castrejón-Gómez², Samuel Pineda¹, José Isaac Figueroa¹, and Ana Mabel Martínez^{1,*}

Abstract

The biological activity of 4 Mexican isolates (SeSIN6, SeSIN8, SeSLP6, and SeSLP8) of the homologous multiple nucleopolyhedrovirus SeMNPV and their effects on the development of the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), were studied. An exotic isolate of SeMNPV from the United States (SeUS2) was used as a reference. Early third-instar larvae were inoculated with 5×10^5 occlusion bodies per mL, resulting in 78% mortality for SeUS2 and approximately 90% mortality for each of the 4 Mexican isolates at 144 h post-inoculation. At 168 h post-inoculation, 100% mortality was obtained in all cases. All of the isolates, including SeUS2, significantly reduced the body weights of *S. exigua* larvae compared with the control larvae; however, at 120 h post-inoculation, the differences among isolates were not significant. All of the isolates reduced the development time of third-instar *S. exigua* larvae (range: 1.2– to 1.6-fold) compared with the control larvae. An independent experiment was performed to determine production of occlusion bodies and mean time to death. Third-instar *S. exigua* larvae (15 h after molting) were inoculated with each isolate using the same concentration mentioned above. The occlusion body production rate was similar among all isolates. The isolates SeUS2, SeSLP6, and SeSLP8 yielded the fastest mortality (range: 187–191 h). Thus, the biological activities of the Mexican SeMNPV isolates were similar to the activity of the exotic isolate, indicating that these indigenous viruses are promising for the biological control of *S. exigua* in Mexico.

Key Words: baculovirus, SeMNPV, biological activity, lethal and sublethal effects

Resumen

Se estudió la actividad biológica y los efectos sobre el desarrollo del gusano soldado *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) de 4 aislados mexicanos (SeSIN6, SeSIN8, SeSLP6 y SeSLP8) de su nucleopoliedrovirus múltiple homólogo (SeMNPV). Se usó como referencia un aislado exótico, originario de Estados Unidos (SeUS2). La inoculación de larvas recién mudadas de tercer estadio con 1×10^6 cuerpos de inclusión per mL resultó en 78% de mortalidad para SeUS2 y aproximadamente 90% para 4 aislados mexicanos a las 144 h post inoculación. A las 168 h post inoculación se observó una mortalidad del 100% en todos los casos. Todos los aislamientos, incluyendo el SeUS2, provocaron un efecto significativo en la reducción del peso corporal promedio de las larvas de *S. exigua* en comparación con el control; sin embargo, a 120 h post inoculación las diferencias entre aislados no fueron significativas. Todos los aislados redujeron el tiempo de desarrollo de las larvas de tercer instar (rango: 1.2–1.6 veces) en comparación con el control. Se realizó un experimento independiente para determinar la producción de cuerpos de inclusión y el tiempo medio de muerte. Para ello, las larvas de tercer estadio de *S. exigua* (15 h después de la muda) se inocularon con cada aislado utilizando la misma concentración descrita arriba. La tasa de producción de cuerpos de inclusión fue similar entre todos los aislados probados. Los aislados SeUS2, SeSLP6, y SeSLP8 causaron una mortalidad más rápida (rango: 187–191 h). Se concluyó que la actividad biológica de los aislados mexicanos del SeMNPV fue similar a la observada en el aislado exótico, indicando que estos virus son prometedores para el control de *S. exigua* en México.

Palabras Clave: baculovirus, SeMNPV, actividad biológica, efectos letales y subletales

Beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), originally from southeastern Asia, is a cosmopolitan pest, and is particularly abundant in North and Central America, Africa, Australia, southern Asia, and Europe (CAB International 2000). This insect is a polyphagous pest of various agricultural crops such as tomato, sweet pepper, beans, cucumber, alfalfa, cotton, and ornamentals (Belda et

al. 1994; Hill 1987; Zheng et al. 2011). Worldwide, Mexico ranks tenth in tomato production and second in pepper production, generating approximately 57.4 million pesos per year (SIAP 2016). *Spodoptera exigua* represents the second most destructive insect pest of sweet pepper and tomato in the northwest and central regions of Mexico, where it can cause damage of 20 to 25% in the cultivation of these

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crops (Gastélum-Luque 2004; Osorio et al. 2008). The control of this pest has involved the intensive use of broad-spectrum insecticides with satisfactory results; however, due to the excessive use of these substances, this insect has developed high levels of resistance to many toxicological classes of insecticides (Moulton et al. 2002; Osorio et al. 2008; Sayyed et al. 2012). In addition, the use of insecticides presents risks of environmental pollution and the reduction or elimination of beneficial insects, among other negative effects. Consequently, effective alternatives for the control of *S. exigua* are needed for the development of integrated pest management programs.

Baculoviruses are natural control agents of a wide range of insect pests. Due to their high specificity and high virulence, some of these viruses are used as bioinsecticides (Hunter-Fujita et al. 1998; Moscardi 1999). The *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) plays an important role in regulating its natural host populations (Gelernter & Federici 1990; Caballero et al. 1992). It has been isolated from different regions of the world (Hara et al. 1995; Hunter-Fujita et al. 1998), and great phenotypic diversity has been reported for this virus (Vlak et al. 1981; Gelernter and Federici 1986; Hara et al. 1995; Muñoz et al. 1999; Murillo et al. 2006).

SeMNPV is of particular interest to the bioinsecticide industry due its high pathogenicity and rapid speed of kill (Smits et al. 1987; Ijkel et al. 1999). This virus constitutes the active ingredient in a number of bioinsecticides sold in different countries for the control of *S. exigua* populations in both open-field (Smits & Vlak 1988; Kolodny-Hirsch et al. 1997) and greenhouse crop systems (Bianchi et al. 2002; Lasa et al. 2007b), including: SPOD-X® (Certis LLC, Columbia, Maryland, USA), SPEXIT® (Andermatt Biocontrol, Grossdietwil, Switzerland), and VIR-EX® (Biocolor, El Ejido, Almería, Spain) (Zamora-Avilés et al. 2017).

However, the selection of indigenous isolates that are suitable for development as biological control agents requires the characterization of the isolates from each geographical region (Figueiredo et al. 2009). In some nucleopolyhedrovirus systems, the native virus tends to be more pathogenic to local populations than to foreign ones (Shapiro & Robertson 1991; Cabodevilla et al. 2011a; Barrera et al. 2011).

In addition to causing direct mortality to the host, baculoviruses also have effects on individuals that survive infection (Duan & Otvos 2001). Sublethal infection presents practical benefits to pest control programs because affected insects are unlikely to cause substantial further damage to plants (Bernal 2013), and experience reductions in development and reproduction (Cabodevilla et al. 2011b). Therefore, the sublethal effects of baculovirus infection can affect the population dynamics of the host (Boots & Norman 2000). Thus, the combination of both lethal and sublethal effects increases the probability of reducing plant damage and abundance of the pest (Duan & Otvos 2001).

The purpose of this study was to determine the biological activity of 4 Mexican SeMNPV isolates, identified previously from infected larvae of *S. exigua* on crops of chili pepper, *Capsicum annum* L., and tomato, *Solanum lycopersicum* L. (both Solanales: Solanaceae), in 2 Mexican states (Zamora-Avilés 2017), and their effects on the development of *S. exigua*. In addition, the production of occlusion bodies and the mean time to death for each isolate were determined.

Materials and Methods

INSECT REARING

Larvae of *S. exigua* were obtained from a laboratory colony established at the Instituto de Investigaciones Agropecuarias y Forestales

(IIAF), Universidad Michoacana de San Nicolás de Hidalgo (UMSNH), Morelia, Michoacán, Mexico, with eggs received from the Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León (Monterrey, Mexico). This colony was maintained in a growth chamber at 26 ± 2 °C, with a 16:8 h (L:D) photoperiod and $75 \pm 5\%$ RH. Larvae were fed a soybean-based semi-synthetic diet as described by Greene et al. (1976) except that pinto beans, casein, and sorbic acid were not included. Adults were fed with a 15% honey solution. Brown paper was provided as an oviposition substrate and was replaced every third day.

VIRUS ISOLATION AND AMPLIFICATION

Four isolates were obtained from *S. exigua* larvae collected from either chili pepper or tomato crops located in the states of Sinaloa and San Luis Potosí, Mexico, in 2006 and 2008. Collected larvae were placed individually in plastic cups (30 ml), provided with an artificial diet, and transported to the laboratory. Larvae showing typical signs and symptoms of baculovirus infection were stored individually at -20 °C after death. For the purification of occlusion bodies, the virus-killed larvae were homogenized in 0.1% (w/v) sodium dodecyl sulfate (SDS) and centrifuged at 90 g for 5 min, and the supernatant was centrifuged at 3,000 g for 10 min. The pellet comprising occlusion bodies was suspended in sterile distilled water for occlusion bodies titration and stored at 4 °C prior to use. The occlusion bodies were counted using a Neubauer improved counting chamber (Brand GmbH & Co KG, Wertheim, Germany) (Hunter-Fujita et al. 1998) under a phase-contrast microscope (400× magnification), with counts performed in triplicate. Fresh occlusion bodies stock was obtained for each isolate by amplification in *S. exigua* fourth-instar larvae via the surface contamination method (Evans & Shapiro 1997) using approximately 1×10^8 occlusion bodies per mL. Forty fourth-instar larvae were fed with a virus-treated diet for 48 h, then reared under standard conditions until death. Occlusion bodies from cadavers were purified as described above.

Samples were identified taking into account the year (2006 or 2008) and the geographical zone of collection (Sinaloa [SIN] or San Luis Potosí [SLP]) and were named as follows: SeSIN6, SeSIN8, SeSLP6, and SeSLP8. The SeUS2 isolate was used as reference (Muñoz et al. 1998) and constitutes the active ingredient of the nucleopolyhedrovirus-based product commercialized as Spod-X® (Certis Europe, Maarssen, Netherlands).

BIOLOGICAL ACTIVITY OF FOUR ISOLATES OF SEMNPV AND THEIR EFFECTS ON LARVAL DEVELOPMENT

This experiment was conducted with third-instar *S. exigua* larvae using a modified droplet bioassay technique (Hughes & Wood 1981). For this technique, starved second-instar larvae were allowed to molt to the next instar over an 8 h period. Groups of 12 recently molted third-instar larvae were fed with droplets of an aqueous virus suspension containing 5×10^6 occlusion bodies per mL and 0.01% (w/v) blue vegetable dye (McCormick® & Company, Inc., Sparks, Maryland, USA). The drops were placed in a Petri dish (about 8 µl per drop), and the larvae were immediately released into the dish, which was then covered with a lid. Previous testing had shown that 10 min was sufficient for the larvae to feed without the drops evaporating. Control larvae were treated with dye solution only. Larvae that ingested droplets within 10 min were individually transferred to cells of a 24-well tissue culture plate (3.6 mL), with each well containing diet, and reared at 25 ± 2 °C. Bioassays were performed independently 3 times ($n = 36$ larvae per treatment).

Larval mortality was determined every 24 h for 7 d (168 h post inoculation). The weights of individual surviving larvae were recorded daily until death. The development of surviving individuals was followed; however, the mean development time was estimated only for those larvae that molted to the fourth instar because very few virus-treated larvae molted to the fifth instar.

The mortality, average weight, and duration of the third instar for virus-treated larvae were analyzed using one-way ANOVA with the program Statgraphics Centurion (Graphic software system; STSC Inc., Rockville, Maryland, USA), with least significant difference (LSD) multiple range tests ($P < 0.05$) used to separate means.

OCCCLUSION BODY PRODUCTION AND MEAN TIME TO DEATH

An independent experiment was performed to evaluate occlusion body production and mean time to death. Third-instar *S. exigua* larvae (9 h after molting) were weighed individually and starved for 6 h. Groups of 25 larvae (15 h after molting) were inoculated with each isolate using the identical concentrations and conditions described above. Initial weights were recorded immediately before inoculation. Bioassays were performed independently on 3 occasions. Forty-five larvae were fed a solution of blue vegetable dye only (control treatment).

Mortality was recorded every 24 h for 9 d (192 h post-inoculation). The weights of individual larvae were recorded before death. Approximately 20 SeMNPV-killed larvae for each isolate were randomly selected for determination of occlusion body production; the occlusion bodies were collected and counted as described by Lasa et al. (2007a). Frozen cadavers were placed individually in 1.5 mL microtubes containing 300 μ l of sterile distilled water, thawed and then filtered through fine steel gauze to remove insect debris. The number of occlusion bodies recovered from each larva was determined using a Neubauer improved counting chamber under phase contrast microscopy at 400 \times (Olympus, BX41TF, Tokyo, Japan). For each larva, counts were performed in triplicate and used to calculate a mean value.

The relationship between occlusion body production and host weight (occlusion bodies per mg) was estimated based on larval weight recorded on the day prior to death (Lasa et al. 2007a). Occlusion body production and weight data were analyzed using one-way ANOVA as implemented with the SPSS 10.0 statistical software package, with least significant difference (LSD) multiple range tests ($P < 0.05$) used to separate means. The values for mean time to death were estimated using mortality data; this analysis was performed using the Generalized Linear Interactive Modeling (GLIM) program with a Weibull distribution specified (Crawley 1993).

Table 1. Larval mortality (\pm SE) of third-instar *Spodoptera exigua* larvae treated with 5 different SeMNPV isolates.

Isolate	Larval mortality (%)			
	Hours post-inoculation			
	72 ^a	96 ^b	120 ^c	144 ^d
Control	0.0 \pm 0.0 a	0.0 \pm 0.0 a	0.0 \pm 0.0 a	0.0 \pm 0.0 a
SeUS2	11.1 \pm 2.8 c	25.0 \pm 8.3 abc	58.3 \pm 0.0 bc	77.8 \pm 7.34 b
SeSLP6	0.0 \pm 0.0 a	8.3 \pm 0.0 ab	52.8 \pm 11.1 b	88.9 \pm 2.8 bc
SeSLP8	0.0 \pm 0.0 a	43.7 \pm 12.8 c	79.3 \pm 8.1 c	94.2 \pm 2.9 c
SeSIN6	5.5 \pm 2.8 b	44.4 \pm 10.0 c	77.8 \pm 14.0 c	94.4 \pm 2.8 c
SeSIN8	0.0 \pm 0.0 a	28.5 \pm 7.2 bc	77.3 \pm 2.3 bc	94.4 \pm 2.8 c

Within the same column, values followed by the same letter are not significantly different from one another ($p = 0.05$).

^a $F = 8.4$; $df = 5,12$; $P = 0.0001$; ^b $F = 4.54$; $df = 5,10$; $P = 0.02$; ^c $F = 14.26$; $df = 5,12$; $P = 0.0001$; ^d $F = 97.38$; $df = 5,12$; $P = 0.0001$.

Results

BIOLOGICAL ACTIVITY OF FOUR ISOLATES OF SEMNPV AND THEIR EFFECTS ON DEVELOPMENT

Isolates SeUS2 and SeSIN6 caused larval mortality at 72 h post-inoculation, whereas the other isolates did not cause mortality until 96 h post-inoculation. By 96 h post-inoculation, isolates SeSIN6 and SeSLP8 began to cause high mortality, although the mortality rate did not significantly differ from that obtained with the reference isolate (SeUS2) and isolate SeSIN8 (Table 1). At 120 h post-inoculation, isolates SeSLP8, SeSIN6, and SeSIN8 had caused the highest mortality (range: 77–79%), although the mortality rates were not significantly different from that obtained with the SeUS2 isolate. At 144 h post-inoculation, isolate SeUS2 had caused significantly lower mortality (78%) than most of the Mexican isolates (Table 1); however, no significant differences were observed among the Mexican isolates (range: 89–94%). At 168 h post-inoculation, mortality was 100% for all of the isolates.

The initial weights of the third-instar larvae at inoculation were not significantly different among the treatments (Table 2). Similarly, the weights at 24 and 48 h post-inoculation were not significantly different among the treatments. The weight ranges were 2.5 to 2.9 at 24 h post-inoculation, and 3.04 to 3.63 at 48 h post-inoculation for all treatments, including the control treatment (Table 2). At 72 h post-inoculation, differences between the isolate treatments and the control were observed only for SeSLP8 and SeSIN8. However, at 96 h post-inoculation, for all of the isolates tested, including the reference isolate (SeUS2), the average body weight of *S. exigua* larvae was significantly reduced compared with that of the control (Table 2), but no significant differences were observed among the isolates (Table 2). Additionally, at 120 h post-inoculation, the body weight of the control larvae (12.53 \pm 0.70 [SE] mg; $n = 33$) was significantly greater than that of the larvae treated with the viruses (range: 3.30 \pm 0.69 to 8.35 \pm 0.89 mg). However, because of the mortality at 120 h post-inoculation, the number of larvae analyzed was very low ($n = 7$ or 8) for 3 of the 5 isolates tested (SeSL8, SeSIN6, and SeSIN8).

All of the isolates resulted in significantly lower development times of third- to fourth-instar larvae of *S. exigua* ($F_{5,204} = 27.3$; $P < 0.0001$) compared with the control treatment (86 h \pm 3.52 h; $n = 34$). The development time of larvae treated with the SeUS2 isolate (107.6 \pm 4.85 h; $n = 35$) was significantly shorter than that obtained with the Mexican isolates (SeSIN6, SeSIN8, SeSLP6, and SeSLP8; range: 131 \pm 3.54 h to 139 \pm 4.46 h; $n = 34$ to 36).

OCCCLUSION BODY PRODUCTION AND MEAN TIME TO DEATH

At 192 h post-inoculation, the mortality caused by the SeUS2 isolate was significantly lower (90%) than that caused by the Mexican isolates, with mortality ranging from 94% to 100% for the isolates SeSL6, SeSL8, SeSIN6, and SeSIN8 ($F_{4,10} = 2.84$; $P = 0.08$). No mortality was observed in the control treatment.

The initial weights of third-instar larvae (15 h after molting) at inoculation were not significantly different among the treatments (range: 4.0–4.5 mg) ($F_{5,356} = 2.09$; $P = 0.06$). However, infected larvae on the day before death had significantly lower weights than those of the controls (Table 3). The larvae with the lowest weights were those treated with isolates SeSIN6, SeSIN8, and SeSLP8 (range: 24–27 mg). The effect of the SeSIN6 isolate was significantly different from the effects of SeUS2 and SeSLP6. Similarly, the effect of SeSIN8 was significantly different from that of the SeSLP6 isolate (Table 3). Although the isolates SeUS2 and SeSIN8 tended to produce the most occlusion bodies, no significant differences were observed in the occlusion body yield per larva (range:

Table 2. Mean weight (mg ± SE) of third-instar *Spodoptera exigua* larvae treated with 5 SeMNPV isolates.

Isolate	Hours post-inoculation (n)*				
	0 ^a	24 ^b	48 ^c	72 ^d	96 ^e
Control	1.33 ± 0.06 a (36)	2.46 ± 0.16 a (36)	3.63 ± 0.19 a (35)	4.29 ± 0.28 c (35)	8.06 ± 0.62 b (33)
SeUS2	1.45 ± 0.08 a (36)	2.59 ± 0.16 a (36)	3.46 ± 0.17 a (35)	3.63 ± 0.20 abc (32)	4.57 ± 0.46 a (29)
SeSLP6	1.49 ± 0.09 a (36)	2.92 ± 0.13 a (36)	3.42 ± 0.16 a (36)	3.77 ± 0.23 bc (36)	4.35 ± 0.39 a (34)
SeSLP8	1.60 ± 0.08 a (36)	2.87 ± 0.15 a (35)	3.21 ± 0.13 a (34)	3.35 ± 0.24 ab (34)	4.12 ± 0.81 a (19)
SeSIN6	1.62 ± 0.07 a (36)	2.88 ± 0.15 a (36)	3.12 ± 0.14 a (36)	3.67 ± 0.22 abc (34)	3.55 ± 0.46 a (20)
SeSIN8	1.45 ± 0.07 a (36)	2.72 ± 0.13 a (35)	3.04 ± 0.14 a (35)	3.07 ± 0.19 a (34)	3.79 ± 0.42 a (25)

Within the same column, values followed by the same letter are not significantly different from one another (LSD test, $p < 0.05$).

^a $F = 1.49$; $df = 5, 208$; $P = 0.078$; ^b $F = 1.58$; $df = 5, 208$; $P = 0.17$; ^c $F = 2.1$; $df = 5, 205$; $P = 0.07$; ^d $F = 3.05$; $df = 5, 198$; $P < 0.01$; ^e $F = 11.35$; $df = 5, 154$; $P < 0.0001$.

*n = number of individually weighed larvae.

3.64–12.9 × 10⁸ occlusion bodies per mL) or occlusion body per mg of larva (1.94–5.4 × 10⁸ occlusion bodies per mL) among the isolates tested (Table 3).

The isolates SeUS2, SeSLP6, and SeSLP8 caused the most rapid mortality, with mean time to death values of 187, 191, and 190 h, respectively (Fig. 1). The mean time to death values for isolates SeSIN6 and SeSIN8 were 193 and 195 h, respectively (Fig. 1).

Discussion

The exotic (SeUS2) and Mexican isolates (SeSIN6, SeSIN8, SeSLP6, and SeSLP8) of SeMNPV caused very similar mortality to third-instar larvae of *S. exigua*. Similarly, previous studies have found non-significant differences in pathogenicity of between 2 SeMNPV isolates of different genotypes (Murillo et al. 2006) and non-significant differences in activity among 3 genotypic variants of SeMNPV (Muñoz & Caballero 2000). Regarding other species of baculovirus, Escibano et al. (1999) observed that the median lethal concentration (LC₅₀) for second-instar *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) did not differ significantly between a *S. frugiperda* multiple nucleopolyhedrovirus (SfMNPV) from Nicaragua (Sf-NIC) and a North American virus (Sf-US). Additionally, Rios-Velasco et al. (2011, 2012) observed that concentrations of 1.2 to 1.9 × 10⁵ occlusion bodies per mL of several Mexican isolates of SfMNPV obtained from different soil samples caused similar insecticidal effects on second-instar *S. frugiperda*.

Small genotypic differences among nucleopolyhedrovirus isolates might have contributed to small differences in biological activity (Smits & Vlask 1988). However, variation in phenotypic traits is associated with genotypic variation in baculovirus populations (Hodgson et al. 2001; Muñoz & Caballero 2000; Murillo et al. 2006; Serrano et al. 2015). High levels of genetic and phenotypic variation were observed in natural

SeMNPV populations originating from groups of virus-killed larvae collected in the field (Muñoz et al. 1999) and from isolates of greenhouse soils (Murillo et al. 2007). This variation among host populations suggests that heterogeneity is important for virus survival (Cooper et al. 2003) and often is associated with phenotypic differences (Hodgson et al. 2001; Cory & Myers 2003; Murillo et al. 2006; Serrano et al. 2015).

In this study, the mortality rates obtained for the Mexican isolates and the SeUS2 isolate tested on newly molted third-instar *S. exigua* larvae were slightly higher than those obtained (80–85% at 192 h post-inoculation) by Cai et al. (2010) for a wild-type SeMNPV and a recombinant virus SeXD1 on third instars of *S. exigua* at a concentration of 5,000 occlusion bodies per larva (about 3.6 × 10⁶ occlusion bodies per mL). Murillo et al. (2006) reported that the inoculation of fourth-instar *S. exigua* larvae with different SeMNPV isolates at a single concentration of 6,453 occlusion bodies per larva (about 1.9 × 10⁶ occlusion bodies per mL) caused between 92% and 97% mortality at 192 h post-inoculation. These mortality rates are very similar to those obtained in this study for third-instar larvae 15 h after molting.

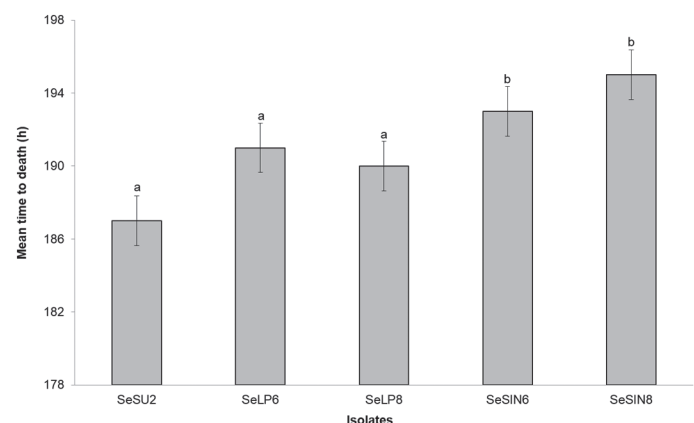
The concentration of isolates used in this study (5 × 10⁶ occlusion bodies per mL for all isolates) resulted in 100% mortality of newly molted third-instar larvae of *S. exigua* at 168 h post-inoculation. However, when a separate group of third-instar *S. exigua* larvae (15 h after molting) was inoculated with the same concentration, 100% mortality at 192 h post-inoculation was obtained with only the SeSIN8 isolate; mortality from the remaining isolates ranged from 90% to 98%. These results are consistent with a study by Arrizubieta et al. (2016) in which the intrastadial age of *Helicoverpa armigera* (Hübner; Lepidoptera: Noctuidae) had an effect on the percentage larval mortality after viral

Table 3. Average weight of *Spodoptera exigua* larvae before death, occlusion body production per larva and occlusion body production per unit weight of larva (mg) following treatment with 5 SeMNPV isolates.

Isolate	Weight of larva (mg) (± SE)	Occlusion bodies per larva (± SE) (× 10 ⁸) ^b	Occlusion bodies per mg of larva (± SE) (× 10 ⁷) ^c
Control	65.8 ± 3.76 d	—	—
SeUS2	38.74 ± 4.6 bc	12.9 ± 6.38 a	3.44 ± 1.4 a
SeSLP6	40.46 ± 4.5 c	8.68 ± 3.29 a	2.59 ± 0.95 a
SeSLP8	27.44 ± 4.5 abc	3.81 ± 0.52 a	1.94 ± 0.35 a
SeSIN6	23.85 ± 8.0 a	3.64 ± 0.49 a	2.41 ± 0.36 a
SeSIN8	24.45 ± 3.1 ab	10.5 ± 3.40 a	5.40 ± 1.61 a

Within the same column, values followed by the same letter are not significantly different from one another (ANOVA, $P = 0.05$).

^a $F = 15.14$; $df = 5, 135$; $P < 0.001$; ^b $F = 1.29$; $df = 4, 94$; $P = 0.27$; ^c $F = 1.61$; $df = 4, 94$; $P = 0.18$.

**Fig. 1.** Mean time of death calculated for third-instar larvae of *Spodoptera exigua*. The numbers above the columns indicate the values calculated for 3 replications. The columns headed by the same letter are not significantly different (Weibull analysis, $\alpha = 1.96$).

infection. The authors of that study observed 85 to 100% mortality in recently molted *H. armigera* larvae across all instars tested (third to fifth instar); however, when the larvae were inoculated 1 d after molting, the percent mortality was significantly lower (21–72%). Therefore, the recently molted larvae were more susceptible to baculovirus infection than were older conspecifics, as also reported in another host-baculovirus system (Grove & Hoover 2007).

The body weights of *S. exigua* larvae exposed to the different isolates decreased by approximately 43% and 56% in the period between molting (before inoculation) and 96 and 120 h post-inoculation, respectively. However, at 120 h post-inoculation, the differences among isolates were not significant. Additionally, the duration of the third instar was increased significantly in larvae exposed to the 4 Mexican isolates relative to the control larvae. Among the isolate-treated larvae, the development time was significantly longer for larvae treated with Mexican isolates than for SeUS2-treated larvae. Similarly, Cabodevilla et al. (2011b) found that when second-instar *S. exigua* larvae were inoculated with 3.8×10^2 occlusion bodies per mL, the larval development time was about 2 d longer than that of healthy insects. When fourth- and fifth-instar *H. armigera* larvae were treated with different concentrations (1×10^6 to 1×10^{10} occlusion bodies per mL) of its homologous nucleopolyhedrovirus (HaSNPV), the development time was longer significantly in most of the treated larvae (range: 16 to 32% and 17 to 27% for fourth and fifth instars, respectively) than in healthy insects (Zhang et al. 2015). Additionally, in the occlusion body-production experiment of the present study, the weight of pupae derived from treated larvae (about 70 mg) was reduced by 30% relative to that of the control pupae (about 100 mg; data not shown). However, these data were not analyzed statistically because the numbers of survivors were few. Reductions in weight of pupae derived from larvae treated with their homologous nucleopolyhedrovirus compared with control pupae also were observed in *S. exigua* (Cabodevilla et al. 2011b) and *H. armigera* (Zhang et al. 2015).

Baculoviruses cause disease primarily in the larval stages, and disease progression and its effects depend on several factors, including the species of virus, host instar age, infective dose, host nutrition, incubation temperature, degree of compatibility of the virus with its host, and the physical characteristics of the larvae (Federici 1977). Most lepidopteran larvae infected with nucleopolyhedroviruses show visible signs of infection 3 to 5 d after viral ingestion, which include behavioral changes (i.e., loss of appetite and cessation of feeding) and reduced mobility (Tanada & Kaya 1993; Fuxa 2004; Hoover et al. 2011). These changes have debilitating effects on the survivors, which are documented widely in lepidopteran-baculovirus systems (Goulson & Cory 1995; Rothman & Myers 1996; Myers et al. 2000; Matthews et al. 2002). In addition to the alterations to development time and the reduction in body weight due to infection that were observed in our study, other sublethal effects caused by baculoviruses have been reported by other authors, including lowered rates of adult emergence (Cabodevilla et al. 2011b), reduced fecundity (Patil et al. 1989; Cabodevilla et al. 2011b), and reduced egg viability (Santiago-Alvarez & Vargas-Osuna 1988; Cabodevilla et al. 2011b).

These debilitating effects of viruses might arise due to the re-allocation of host energy reserves to combat the disease (Bong & Sikorowski 1991) and hormonal changes induced in the host (O'Reilly & Miller 1989). Recently, Zhang et al. (2015) determined that virus infection might suppress the growth of host insects by disturbing the hormonal balance via influence on the viral *Egt* gene, which encodes an enzyme that modifies a hydroxyl group on 20E, thereby inactivating the molting hormone and resulting in a delay in or absence of molting in infected larvae (O'Reilly et al. 1992; Chen et al. 1997; Slavicek et al. 1999).

Generally, the most productive isolates tend to be those with lower virulence (Barrera et al. 2011; Cabodevilla et al. 2011a; Murillo et al. 2006). However, in this study, occlusion body production did not differ significantly between isolates with slow (SeSIN8) and rapid (US2) speed of kill, consistent with the small difference in mean time to death values (about 8 h) between these isolates. The occlusion body productivity per mg of larva (range: $2\text{--}5 \times 10^7$) generally was consistent with that observed for lepidopteran nucleopolyhedroviruses (range: 9.2×10^6 to 4.2×10^7 occlusion bodies per larva) (Claus & Sciocco de Cap 2001). Regarding the number of occlusion bodies per larva, the values obtained in the present study (range: $3\text{--}13 \times 10^8$ occlusion bodies per larva) are similar to those obtained by Murillo et al. (2003) (2.99×10^8 occlusion bodies per larva), Elvira et al. (2013) (about 8×10^8 occlusion bodies per larva), and Cabodevilla et al. (2011a) (2.99×10^8 occlusion bodies per larva) for recently molted, fourth-instar *S. exigua* larvae inoculated with different wild-type SeMNPV isolates. The similar results among studies might be due to our use of third-instar *S. exigua* larvae (15 h after molting), which have a higher body weight than earlier-instar larvae. Arrizubieta et al. (2016) observed no significant difference at higher body weight than earlier-instar larvae in occlusion body production per larva between third instars and recently molted fourth-instar *H. armigera* larvae (24 h post-molting) inoculated with their homologous virus (HaMNPV).

Compared with the exotic isolate used as a reference in this study, the Mexican SeMNPV isolates were competitive in their lethal and debilitating effects. Clearly, the selection of an isolate as a biocontrol agent requires that geographical variants be tested on insect populations from the locality in which the control program is established. Studies are being conducted to determine the genetic characteristics of the Mexican SeMNPV isolates tested in this study. Additional studies are required to further determine the biological characteristics of these viruses and to determine their efficacies in the greenhouse and field.

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