TESIS DE MÁSTER

Influencia del sexo en la transmisión vertical del nucleopoliedrovirus de *Spodoptera exigua* (SeMNPV-AI1).

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Curso 2011-12

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ABSTRACT

Baculovirus ecological cycle involves two ways of transmission: horizontal transmission between congeners mediated by occlusion bodies and vertical transmission when viral genomes pass on from parents to offspring. Recent studies reported the effective vertical transmission of the Nucleopolyhedrovirus of *Spodoptera exigua* (Baculoviridae, SeMNPV) in host populations of covert crops in Almería (Spain). The production of infected progeny from field-caught healthy females, suggests both sexes were involved in the viral transgeneral transmission. In the present study, sublethal infections were established to obtain covertly infected adults and setting a crossing schedule between healthy and virus-treated adults; we demonstrate that both parental adults were capable to transmit SeMNPV infection to a subsequent generation. Females seem to be more consistent in passing along the infection based on the viral load measured in the offspring using qPCR. Viral load per insect average of descendants from any mating group was similar independently of the gender of infected parental and also female and male descendants resulted equally infected. Egg decontamination treatment did not affect viral detection indicating that transmission was likely transovarial.

KEY WORDS: Gender, SeMNPV, *Spodoptera exigua*, transovarial, Vertical transmission.

RESUMEN

El ciclo ecológico de los baculovirus comprende dos vías de transmisión: la horizontal que se produce entre congéneres y la vertical, que implica el paso de genomas virales de los parentales a la progenie. Recientemente se ha estudiado la efectiva transmisión vertical del Nucleopoliedrovirus de *Spodoptera exigua* (SeMNPV-Al1) en una población de su húesped de los invernaderos de Almería (España). La detección de progenie infectada de hembras sanas, sugiere la necesidad de determinar el papel que juegan ambos sexos en la transmisión del virus. En el presente trabajo se establecieron infecciones subletales para obtener adultos con infecciones encubiertas y utilizando un esquema de apareamientos entre adultos sanos e infectados se verificó que la transmisión del virus es posible vía paterna o materna. La vía materna parece más constante en su respuesta de acuerdo a la medición de la carga viral obtenida en la descendencia (qPCR). El tratamiento de desinfección de la puesta no afectó a la detección de ADN viral en la descendencia, lo que sugiere una transmisión *transovo.* La carga viral por insecto fue similar independientemente del sexo de los parentales y la descendencia masculina y femenina se vio afectada de igual manera por la infección.

PALABRAS CLAVES: Género, SeMNPV, *Spodoptera exigua*, Transmisión vertical, transovárica.

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INTRODUCTION

Baculoviruses are the most extensively studied arthropod-specific virus due to their extremely high virulence to worldwide insect pest and for their good genomic characteristics for mass-production of recombinant proteins. Members of this group have been mainly isolated from insects of the Lepidoptera, Hymenoptera and Diptera orders. Because of their high specificity, persistence and safeness to non-target organisms (including humans), they have been developed as insect pest control agents against important pest for crops and forests protection (30). The genome is formed by a single circularized doubled-strain molecule of DNA, and ranges from 80 to 180Kbp which encode around 90-180 genes (11). DNA is packaged in rod-shaped nucleocapsids conforming single or multiple virions which are typically occluded into a proteinaceus matrix. The occlusion bodies (OB) presents two morphological types attending to the number of virions contained: the multiple form named nucleopolyhedrovirus (NPV) and the single form named granulovirus (GV) (35). Taxonomically, a recent revision proposed by the International Committee for the Taxonomy of Viruses (ICTV, 2009) divided the Baculoviridae family in four genera: i) *Alphabaculovirus*, which includes lepidopteran-specific NPV; ii) *Betabaculovirus*, which takes in lepidopteran-specific GV; iii) *Gammabaculovirus*, including hymenopteran-specific NPV and iv) *Deltabaculovirus*, which contains dipteran-specific NPV (to date, comprises only *Culex nigripalpus* NPV) (22, 29).

It is well-known that dynamics of baculovirus population involve two transmission pathways to colonize and survive in the host. Occlusion bodies (OB) are responsible of the between larvae (horizontal) transmission, thought to be major pathway of baculovirus transmission. Infection cycle begins with the ingestions of OB, naturally occurring in phyllosphere and soil, by a susceptible host. After ingestion, the proteinacious matrix of the OB, is dissolved by the alkaline pH of the midgut; releasing the virions to the lumen (occlusion derived virion-ODV). These particles are capable to pass through peritrophic membrane and fuse by specific receptors to the columnar cells membrane. The naked nucleocapside is injected into the cytoplasm, and directed towards the nucleus, to set off a first replication cycle. Alternatively, some nucleocapsides pass through the cytoplasm and go to the basal part of the cell, where they are enveloped by the cellular membrane and budded to the periplasmic space (budded virus-BV). Hence, the BV starts the secondary infection, moving to and infecting other tissues through the hemocele and the tracheoles. OBs assembly results in a hypertrophic nucleus that eventually leads to cellular lysis and tissues disruption. The cycle is closed few days after with the larval death, and the OBs release to the environment, due to the weakness of tegument destroyed by the combined action of a viral-encoded quitinase and cathepsine, and external mechanical forces.

Vertical transmission has been proposed as a survival strategy to overcome periods of host population scarcity and to facilitate the virus dispersal to geographically spaced niches (10). This pathway requires the transmission of the virus from infected parents to offspring, being in a transovarial or transovum manner. Transovarial transmission requires the virus passing within the eggs, either as virus particles or genome only; while in transovum, viral particles are located on the egg surface and neonates get infected when they ingest the chorion (26, 40). Vertical transmission also implies the persistence of virus in the insect larvae, which has been described in one of two forms: latent, when the virus is essentially dormant (neither replication nor transcription) and persistent when the viral genes are expressed at low levels (5). Persistent infections of NPVs often have a biological cost: weakening effects has been observed on the host population, such as lower developmental rates, lower pupal and adult body weight and reduced reproductive capacity (8, 15, 17, 26, 33), although sometimes beneficial effects have been detected (37). Interestingly, the baculovirus manage to transmit themselves through several generations (9, 38), resembling the behavior of other viruses infecting humans (rabies and Epstein-Barr (6)) or insects (Drosophila Sigma virus, Drosophila C virus or Drosophila S virus) which adopted different strategies for avoiding of clearance by host immune system(23).

Covert infections caused by nucleopolyhedrovirus have been reported in a wide variety of lepidopteran species including *Spodoptera* complex (9, 12, 36), *Mythimna separata* (34), *Mamestra brassicae* (18, 19), *Lymantria dispar* (32), *Pseudoplusia includens* (26), *Bombyx mori* (24), *Trichoplusia ni* (13, 14) and *Operophtera brumata* (4), showing differences in transmission rates, sublethal effects and persistence through different insect stages; likewise its occurrence in field-sampled (4, 8, 26, 40), and laboratory-reared populations (13, 14, 26, 31). Due to the lack of methods of high sensibility for detection and quantification of viral genomes in covert infections, first approaches were based on observation of NPV symptoms in some individuals of apparent free-virus populations after stress or in their offspring (26). As the molecular techniques for transcripts detection (mRNA) were developed, persistent infections caused by baculoviruses had been described in different hosts like such as *Mamestra brassicae* (L.) (18, 19), *Plodia interpunctella* (Hübner) (7), *Spodoptera exempta* (Walker) (40) and *S. exigua* (9). Also latent infections has been reported for all developmental stages of asymptomatic individuals of *S. exigua*, confirming the virus is detectable at any time in the host life cycle (31).

Cabodevilla et al (8) demonstrated that vertical transmission of *S. exigua* nucleopolyhedrovirus (SeMNPV) is a common feature in field populations of *S. exigua* in Almeria. In their study, a number of vertically transmitted genotypes, were isolated and characterized for their insecticidal properties. Among them, the SeVTAl1 genotype showed great capacity to induce persistent infections compared to other genotypes associated to the horizontal transmission pathway, and the prevalence of an induced covert infection was detectable even five generations after establishment (9). Unexpectedly, field-caught gravid females were observed

to produce virus-infected offspring even though no evidence of the infection was seen in these females using the most sensitive detection methods available. This led us to suspect that both sexes may contribute to vertical transmission of the pathogen. However, the differential prevalence of covert infection between males and females, suggests a possible gender effect on the transmission mechanisms.

The *Spodoptera* complex (Lepidoptera: Noctuidae) consists of several species of this lepidopteran, which have shown high damage potential and causes several economic losses in crops. These insects are polyphagous, attacking a wide variety of field plant species like corn, soybean, cotton, tomato and other crops of economic importance (3). *Spodoptera exigua*, *S. frugiperda*, *S. litura* and *S. littoralis* have been reported as the most important pests included in this complex (2, 3). In Almeria (Spain), some authors have addressed *S. exigua* as the major pest for sweet pepper, tomato, aubergine, courgette, melon and watermelon crops under greenhouse conditions, importantly affecting crop production (1). Recently, a NPV-based bioinsecticide has been developed and successfully applied to control *S. exigua* larvae in sweet pepper greenhouses (27). The aim of the present study is to determine the effect of gender on the transmission efficiency of SeMNPV leading to sublethal infections in the subsequent host generation. Improving of knowledge on the factors affecting vertical transmission mechanism may contribute to the development of optimal strategies for the use of NPV-based bioinsecticides.

MATERIALS AND METHODS

Insects and virus

A virus-free *S. exigua* laboratory colony was obtained from Andermatt Biocontrol AG (Grossdietwil, Switzerland) and reared on artificial diet. Insects were maintained at a constant temperature (25±1°C), relative humidity (RH; 50%±5%), and photoperiod (16-h/8-h light-dark cycle) in the insectary facilities of the Universidad Pública de Navarra, Pamplona, Spain. The *S. exigua* nucleopolyhedrovirus genotype VT-SeAl1 was used in all the experiments. This genotype was previously isolated from a sublethally infected culture of insects collected in the greenhouses of Almeria (Spain), and it has been associated to an efficient vertical transmission (8, 9).

Bioassays

To determine gender influence on vertical transmission of the VT-SeAl1, groups of adults sublethally infected or virus-free adults were required. In order to obtain sublethally infected adults we followed methodology described by Cabodevilla et al (9). Briefly, groups of 200 premolted *S. exigua* fourth instars were starved overnight and once molted, orally droplet-fed with a suspension of 9×10^3OB/ml (DL_{50} calculated (9). Once they drank the virus suspension,

the larvae were individually placed in 25-ml plastic cups perforated for ventilation and provided with artificial diet. In parallel, a group of 100 molted *S. exigua* fourth instars were treated in the same conditions but without OB suspended. Larvae that did not succumb to NPV disease were reared through to pupation at 25±2°C and 50% ±5% RH. Pupae were sexed by looking at the final segment of the abdomen and then reared to adults in separate groups in function of their sex and treatment.

Once the adults emerged, we set up the following mating plan involving four groups: i) healthy females (HF) × healthy males (HM); ii) healthy females (HF) × infected males (IM); iii) infected females (IF) × healthy males (HM); and iv) infected females (IF) × infected males (IM). Five adult pairs were placed in paper bags for oviposition provided with a wet cotton ball at standard rearing conditions (25±1°C and 50% ±5% RH). Eggs batches from each treatment group were harvested and the adults frozen at -80°C for subsequent analysis (F0 generation). Experiments were carried out five times.

Eggs decontamination

The eggs were divided into two samples and either soaked in a 0.25 ppm NaClO solution (surface decontaminated group) or distilled water (non-decontaminated group) for five minutes. Then allowed to dry and placed in a Petri dish with artificial diet until the larval hatching. Twenty-five neonates were individually reared through to adult on semi-artificial diet. Newly emerged adults were frozen and stored at -80°C until total DNA extractions (F1 generation). The experiments were carried on five times.

DNA extractions and quantitative PCR (qPCR)

Total DNA was extracted using MasterPure Complete DNA Purification kit (Epicentre Biotechnologies) standard protocol for tissue samples. Abdomens of frozen adults were dissected, and sexed by the observation of the external genitalia. Then the complete abdomen was placed in a 2 ml tube with ceramic beads and 300 µl of Tissue and Cell Lysis Solution with 1µl of 50µg/µl Proteinase K added. The tissue was grounded using the MP FastPrep-24 tissue in a cell homogenizer at 4.0 m/s for 20 seconds. Subsequently, the mixture was incubated at 65°C for 15 minutes at a constant 1100 rpm orbital shaking. One microliter of 5µg/µl RNasa was added to the mixture and then incubated at 37°C for 30 minutes. Debris was pelleted by adding Protein Precipitation Reagent and centrifugation. DNA was precipitated using cold isopropanol, washed twice with 70% ethanol, resuspended in 20 µl deionized water and stored at -20°C. Blank extraction samples containing only water were processed in parallel to detect cross-contamination during the extraction process.

Quantitative PCR based on SYBR fluorescence was carried out in an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems) in 96-well reaction plates. Amplification were

performed in a total reaction of 10µl containing 5 µl of SYBR Premix Ex Taq (2x), 0.2 µl of ROX Reference Dye (50x), 0.2 µl of both forward and reverse primers (10 pmol/µl) (Table 1), and 1 µl of DNA template. Three non-template reactions were included in each run and a standard curve in duplicated for determining the efficiency of the reaction. The qPCR protocol consisted of an initial denaturation at 95°C for 30 s, followed by 45 amplification cycles of 95°C for 5 s, 60°C for 30 s, and finally added a dissociation stage of 95°C for 15 s, 60°C for 15 s, 95°C for 15 s. Data acquisition and analysis were handled by Sequence Detector System v 2.2.2 software (Applied Biosystems, 2004). Reaction parameters and conditions were optimized in a previous study (Unpublished data), including primer design for specific viral amplification on a total DNA of genomic background from insect cells (DNApol149-Fw: 5'-CCGCTCGCCAACTACATTAC-3'; DNApol149-Rv: 5'-GAATCCGTGTCGCCGTATATC-3').

A standard curve was performed to consistently estimate DNA quantities per sample by extrapolation of Ct values. SeMNPV DNA was quantified using a spectrophotometer (eppendorf BioPhotometer plus) and then serially diluted in sterile MilliQ water (dilutions corresponding to: 1×10^{-2} , 1×10^{-3} , 5×10^{-4} , 1×10^{-4} ,5×10^{-5} , 1×10^{-5} , 5×10^{-6} , 1×10^{-6} , 5×10^{-7} , 1×10^{-7} ng/µl) A total of seven replications of the DNA dilutions were performed and the average of Ct values obtained for each point was calculated and then a linear regression fitted.

Every DNA sample was performed in triplicate and the specificities of PCR products were monitored by analyzing amplification profiles and the corresponding dissociation curves. The detection limit or cut-off value was considered the lowest dilution showing the correct amplification curve and a peak at the expected melting temperature (84°C) for the dissociation curve. Quantified viral DNA was normalized based on the total DNA concentration for each sample, measured using a NanoDrop 2000. In order to express the detected amount of viral DNA in number of copies of viral genomes, the values of concentration of viral DNA were transformed into number of copies using SeMNPV length of 135611 bp (20) according to the following equation

$$\textbf{Number of genomes} = (\text{DNA (ng)} \times 6.022\times10^{23}) / (135611 \text{ bp} \times 1\times10^{9} \times 650)$$

Statistical analysis

Non-parametric tests were applied to the viral DNA quantification data, given the lack of normality and heterocedasticy. The comparison between the treatments was carried out using the Kruskal-Wallis rank analysis, with the Bonferroni's test as post-Hoc tool. Also, the results of the qPCR were analyzed in a factorial form, grouping the results according to the variable evaluated. Four factors were proposed as categorical variables with two levels: i) parental male and female: free from virus (Healthy) or virus infected (Infected); ii) surface decontamination of eggs treatment (submitted or no submitted) and iii) offspring sex (male or female) assuming the

size was unpredictable due to the inner sex ratio of the offspring. The response evaluated was the quantity of viral DNA per micrograms of total DNA in each sample and the main factors were submitted to analysis using the Kruskal-Wallis analysis of variance on ranks. All the tests were carried out with a confidence level of 95% using the free software R v 2.0.14. (The R Foundation for Statistical Computing, 2011). Graphics were done using software SigmaPlot for Windows v 11.0 (Systat Software Inc. 2008).

RESULTS AND DISCUSSION

NPV-induced mortality and qPCR parameters

Mortality response of larvae in bioassay was checked to confirm the induction of sublethal infections (Table 1). Percentages of mortality associated to viral infection ranged from 44.7% to 76.9%. According to the administered OB dose to larvae a mortality of ~50% was expected (8). Cabodevilla et al. demonstrated that prevalence of sublethal infections is in some way dose-dependent. They found higher frequencies of sublethally infected adults as the administered OB dose was more concentrated. In that study, NPV-induced mortality above 50% resulted in ~80% of adults infected (9). Regarding to this, in the present study high prevalence of infected individuals in offspring could be anticipated. As expected, no viral mortality was detected in mock infected treatments.

Table 1. Percentages of mortality due to NPV infections.

REPLICATE	NON-INFECTED		INFECTED	
	N	% NPV MORTALITY	N	% NPV MORTALITY
1	100	0	152	44.7
2	100	0	189	60.3
3	100	0	188	52.7
4	100	0	173	72.8
5	100	0	117	76.9
Mean ± s.d.	-	0	-	61.5±13.5

** viral-induced mortality was calculated using only the surviving population, the number of deaths caused by agents different to NPV have been discarded.*

In order to establish the parameters for the qPCR reaction, a calibration curve was fitted using concentration of viral DNA (Figure 1). The value of slope of -3.215 obtained for equation confirmed the goodness and high efficiency of the reactions, given that slope values of -3.32 have been related with the 100% of the reaction efficiency (21). Because of the need of a cut-off value, the last concentration detected that kept the linearity of the regression was chosen as the limit of detection for this trial. This value was set as 33.4 cycles and all the samples that

showed higher Ct values were treated as negative, while samples with lesser number of cycles, and showing the correct amplification profile in the dissociation curve, were considered as positive and quantified.

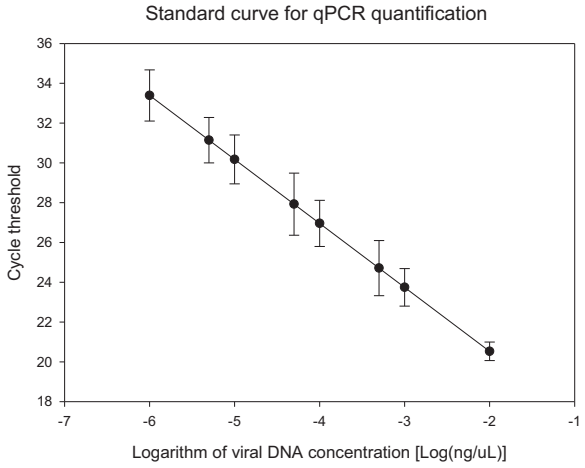

Figure 1. Standard curve for qPCR for quantification of the DNA load (Ct=-3.215 x + 36.07; R^2=0.999). Data are logarithmic transformed. Bars represent standard error obtained for each experimental point.

Establishing sublethal infection

Adults survivors to the virus challenged were used to set up four mating groups: i) healthy females (HF) × healthy males (HM); ii) healthy females (HF) × infected males (IM); iii) infected females (IF) × healthy males (HM); and iv) infected females (IF) × infected males (IM). Parental insects for each of the four mating groups were checked for virus to determine sublethal infection levels. QPCR positive individuals were more abundant in virus challenged insect groups (χ^2=17.190; *d.f*= 1; *p-Value*=0.0004), some non-expected positive individuals were detected in mock-treated individuals (Table 2). Twelve out of the 100 samples from insects that had been non-exposed to virus were detected as positives. False positives found when virus-free individuals were mated could be explained due to cross-contamination of the sample or the presence of artifact products during qPCR reaction.

Venereal transmission

Another plausible explanation for qPCR positive between non-exposed to virus insect groups could be the venereal transmission. Such route of infection has been described for three different insect virus during mating: a Nudivirus in *Oryctes rhinoceros*, a Parvovirus in *Aedes albopictus* and two NPV in *Heliothis zea* and *Hyphantria cunea* (25). Furthermore, Hamm and colleagues (16) identified a non-occluded baculovirus in *H. zea*, that was capable of passing

from infected males to females after a unique copulation, through waxy secretions from the tip of the abdomen. Regarding qPCR positives detected on free-virus groups (5/50) were as many as the numbers in the apparently healthy groups mated with infected insects (HF=4/25 and HM=3/25; Table 2), it seems unlikely that venereal infections may occur (χ^2=0.300; *d.f*= 1; *p-Value*=0.584). However, further experiments need to be undertaken to clarify whether SeMNPV transmission during intercourse is possible.

Table 2. Number of qPCR positive of parental adults for mating groups

REPLICATES	MATING GROUPS (F0)							
	HM × HF		IM ×HF		HM×IF		IM ×IF	
	HM	HF	IM	HF	HM	IF	IM	IF
1	0	0	5	1	2	5	5	4
2	1	0	5	2	0	5	4	3
3	2	1	4	1	1	3	2	5
4	1	0	3	0	0	3	3	1
5	0	0	0	0	0	4	0	0
TOTAL*	4	1	17	4	3	20	15	13
PERCENTAGE	16	4	68	16	12	80	60	52
Median Viral Load (× 10^{-6}ng viral DNA/µg total DNA)	0.31	0	2.4	0.11	0	2.0	3.8	1.5

*Numbers of individuals used and analyzed per treatment = 50 HM= Healthy male; HF= Healthy female; IM= Infected Male; IF= Infected Female

Viral transmission to the offspring

The presence and abundance of virus in the subsequent generation of those virus-treated insects was determined for mating groups in order to relate viral abundance in offspring to parental gender. Both male and female parental adults were capable to transmit the infection to descendants (Table 3). The prevalence of sublethally infected insects was similar for mating groups involving infected adults (Bonferroni's post-Hoc test; α=0.05), but all these three groups presented statistical differences respect to healthy controls (χ^2=8.422; *d.f*= 3; *p-Value*=0.038).

Quantification of viral load in the offspring (F1) was estimated and normalized by total DNA for each sample. Amounts of viral DNA were significantly different between mating groups (χ^2=8.794; *d.f*= 3; *p-Value*=0.032). Post-Hoc Bonferroni's test (α=0.05), resulted in grouping the treatments into two levels (Table 3). Mating pairs IF x IM and IF x HM were statistically similar one to another but different from HF x HM, while HF x IM presented an intermediate response. This result indicates that both mating groups involving female infected parental transmit higher viral loads to their offspring than healthy controls. The response generated in the offspring from infected male, when coupled with healthy female, showed a high variability and could not be distinguished to the offspring from either the control healthy parental or the one-part infected mating groups. Interestingly both the prevalence and the viral load for mating pairs

involving infected females result in higher values, pointing out a trend of females being more efficient than males in transmission.

Table 3. Viral detection in offspring for mating groups

MATING GROUP	MEDIAN PREVALENCE (%)	MEDIAN VIRAL LOAD* (ng viral DNA/ µg total DNA)	IQR**
HF x HM	6.43b	3.09×10^{-8} b	6.55×10^{-8}
HF x IM	28.26a	4.22×10^{-7} ab	3.15×10^{-7}
IF x HM	42.55a	8.73×10^{-7} a	6.11×10^{-7}
IF x IM	36.29a	6.49×10^{-7} a	2.39×10^{-7}

*N= 5. **Inter-Quartile Range. HM= Healthy male; HF= Healthy female; IM= Infected Male; IF= Infected Female. Values followed by different letters indicate significant differences between groups by Post-Hoc Bonferroni's test (α=0.05)

Subsequently the exploration of the effect caused by each main factor was carried out. The male parental effect did not significantly affect the response (χ^2=2.723; *d.f*=1; p =0.098), while the female parental did (χ^2=13.827; *d.f*=1; p =0.0002). This result is in line with the analysis stated above, the treatments involving infected females exposed to have a greater and stable effect on the quantities detected, while the treatment where the only donor of virus was the male, had a greater variability. Biologically, this implies that the infection could be passed through males or females to the offspring, but the females seem to be more consistent in transmission than males. For the vertically-transmitted Reovirus DSV (*Drosophila S virus*), a causative agent of special phenotypes in SimEs strain of *Drosophila simulans* (28), the transmission of viral particles is typically carried out inside of the oocyte, likely due to the differences in size and activity between male and female gametes. Our results are in line with those for the reovirus DSV, indicating that transmission rates are greater in females than males of *D. simulans* despite of viral activity replication occurred either inside testes or ovaries. It is worth noting, that variation of the response generated in infected male (IM) x healthy female (HF) treatment in our study, indicates that males were capable to transmit the infection but the response was strongly variable. More recently, studies conducted focus on the transmission of BmNPV in *Bombyx mori* (24) and the *Plodia interpunctella* GV demonstrated that both sexes were involved in vertical transmission (5). Interestingly, they found viral particles in either testis or ovaries, confirming the presence of the virus in gonads of sublethally infected individuals by histological observation (24) or by viral transcript detection (5, 24). For the BmNPV study the offspring was observed for NPV-mortality but they did not find differences between the prevalence of the virus, independently of the virulent source of contamination (male or female). They established differences between the pathways of transmission preferred for the virus, according to the source of contamination. Mating pairs with infected female, generated

higher mortalities in first instar offspring (78%) than the corresponding obtained in treatments where only male was the donor of virus (57%). This fact was associated to a preferred transovarial transmission of the virus thereby females gave the virus or a preferred transovum transmission of virus with male as the donor. In this study we would expect transovarial transmission more effective than transovum regarding our results.

Vertical transmission of virus is mainly associated with females, as the non-mobile gamete, the oocyte, is not inactivated by the presence of some exogenous material, like virus particles. For the well-known vertically transmitted insect virus, the Drosophila Sigma virus, two different kinds of transmission have been described, the so-called "stabilized transmission" and the "non-stabilized transmission". In the first type, almost all the offspring is infected, and the viral load is relatively constant (13). In the second type, not all the offspring is infected, and the viral load is far more variable. Males are able to transmit only in a non-stabilized way, while females transmit in both ways. The "stabilization" strategy required the female to have acquired the virus by vertical transmission. By comparison, the percentage of transmission observed in our study is not statistically different between males and females infected parental groups (see Table 3, 28% for IM and 42% for IF). A strategy similar to the "stabilization" could not be detected in our conditions, as we have limited the study to the F1. However, we observed variability in transmission rates of the male lineage to passing on the virus to the subsequent generation. Hence, lower titer of virus in sperm than in oocytes of sublethally infected adults may be expected for insect suffering sublethal infection of baculovirus.

The average of viral load estimated per infected insect was also compared for treatments in order to evaluate weather individual's results in similar levels of infection between mating groups (Table 4). Median values of viral load were similar (Kruskal-wallis test; χ^2=6.22; *d.f*=3; p-value=0.101) and ranged from 8.71 x 10^{-7} to 1.48 × 10^{-6} ng viral DNA/ µg total DNA, which equates from 4 to 34 SeMNPV genomes per µg of DNA. Therefore the quantity of viral DNA per sublethally infected insect was not influenced by the source of the infection (male, female or both).

Table 4. Viral load per insect (ng viral DNA/ µg total DNA) for mating groups.

MATING GROUP	MEDIAN*	IQR**
HF X HM	8.71 x 10^{-7}	6.45 x 10^{-7}
HF x IM	1.48 × 10^{-6}	8.00 × 10^{-7}
IF x HM	2.01 × 10^{-6}	6.30 × 10^{-7}
IF x IM	1.68 × 10^{-6}	2.16 × 10^{-6}

*N= 5. **Inter-Quartile Range. HM= Healthy male; HF= Healthy female; IM= Infected Male; IF= Infected Female. Values followed by different letters indicate significant differences between groups by Post-Hoc Bonferroni's test (α=0.05)

Effect of surface decontamination on transmission

Surface decontamination had no effect neither on the viral load quantified for the offspring from infected mating pairs (Non-decontaminated eggs median= 5.842 X 10^{-7} ng viral DNA/ µg total DNA; IQR= 2.202 X 10^{-7}; decontaminated eggs median= 5.266 X 10^{-7} ng viral DNA/ µg total DNA , IQR= 5.391 X 10^{-7} ; χ^2=0.0009; *d.f*=1; p-value=0.9766), nor in the viral load per insect (Non-decontaminated eggs median= 13.98 copies of viral genome/ µg total DNA per insect IQR= 1.24; decontaminated eggs median= 11.68 copies of viral genome/ µg total DNA per insect; IQR= 1.90; $\chi 2$=1.32; *d.f*=1; p-value=0.31). This result suggests that transovarial transmission represent the most likely pathway for the virus to infect descendants. Two main mechanisms for the virus to be transmitted to offspring have been described, transmission via contamination of the chorion, thereby infection when the neonate larvae eats the chorion at hatching; and transmission per ovum, when the virus particles, or the virus genome, is carried inside the egg. Both strategies for vertical transmission have been reported for different species of NPV-host system in previous studies, based on the offspring NPV-mortality but not on molecular techniques of viral detection (15, 26, 33, 34). Our results are in agreement with more recent studies made with SpexNPV on *S. exempta* reporting that surface decontamination of eggs did not affect the detection of the virus in the offspring of infected insects (39, 40).

Virus distribution according to gender offspring

We investigated whether the offspring was more likely to result infected according to gender. We observed that males (Median= 5.321 X 10^{-7} ng viral DNA/ µg total DNA; IQR=7.990 X 10^{-8}) and females (Median= 5.904 X 10^{-7}ng viral DNA/ µg total DNA; IQR=4.875 X 10^{-7}) resulted equally infected (χ^2=1.7129; *d.f*=1; p-value=0.1904), thus infection produced in the offspring was not biased by the sex of the neonate insect. Additionally, we compared the average of viral load per insect according to gender. Median viral load did not differ between males (13.48 copies of viral genome/ µg total DNA per insect; IQR= 4.74) and females (12.90 copies of viral genome/ µg total DNA per insect; IQR= 2.12) (χ^2=0.0109; *d.f*=1; p-value=0.9168). This is in line with those results previously found in field studies, where the prevalence of sublethal infection caused by NPV in field-caught adults was similar between males and females (8).

CONCLUSIONS

- This study demonstrates that vertical transmission of the SeMNPV is possible through either *S. exigua* parental male or female adult, despite female tend to transmit the virus more consistently to the offspring. Biparental vertical transmission may contribute to baculovirus rapidly spread to insect population, even if the infection is costly to its host.

- Venereal transmission between mating pairs seems no likely to occur, although some adults from groups non-exposed to the virus were detected positive. Further researching is needed to demonstrate if venereal transmission of the virus allows horizontal transmission in this species.
- The main pathway of transmission of the SeMNPV is transovarian as surface decontamination of eggs did not affect infection levels in the offspring.
- Finally, the distribution of the virus in the offspring is not biased by gender, being equal likely to be infected male than female embryos.

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