Philippine Journal of Science
149 (3-a): 887-896, October 2020
ISSN 0031 - 7683
Date Received: 20 May 2020

Cross-infectivity of a Putative *Spodoptera picta* Nucleopolyhedrovirus to *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae)

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A putative nucleopolyhedrovirus (NPV) from a field-collected local *Spodoptera picta* population was isolated for the first time. Its pathogenesis was described in *S. picta* larvae and was tentatively assigned the name *Spodoptera picta nucleopolyhedrovirus* or SppiNPV. With the objective of utilizing this SppiNPV as a biological control agent, its cross-infectivity to *S. litura* – a very important agricultural pest in the country – was evaluated. Third instar *S. litura* larvae were infected with approximately 100 occlusion bodies (OBs) of SppiNPV using artificial diet- incorporation. SppiNPV infection in *S. litura* was clearly observed as the 4th instar larvae exhibited pinkish coloration, a characteristic sign of NPV-infection. In addition, the integument of the mildly-infected larvae easily twisted when touched and often expelled slimy fluid from their mouth. At the advanced stage of infection, larvae became extremely fragile resulting in cuticles that were easily ruptured. At 6 d post-infection (dpi), 20% mortality was observed in the SppiNPV-treated *S. litura*. At 16 dpi, all the cutworms in the control set-up emerged as normal adults, while only three cutworms (10%) in the NPV-treated larvae emerged as adults but with short malformed wings and abnormally soft and fragile abdomen. The effect of SppiNPV on the weight gain of *S. litura* larvae was also monitored at 4 and 6 dpi. Although significantly higher weight ($F = 5.09$; $df = 58$; $P > 0.0278$) was recorded in the untreated cutworms than the NPV-treated cutworms at 4 dpi, weight gain did not significantly differ in SppiNPV-infected and untreated larvae. Overall, a significantly higher mortality of 46.67% ($F = 25.37$; $df = 58$; $P > 0.0001$) was recorded in SppiNPV-infected cutworm larvae. Further studies to characterize this novel SppiNPV, increase its virulence to *S. litura* through the continuous passage in *S. litura*, and optimize the amount of OB to be applied to ensure 100% mortality are recommended so that it can be used as a biological control agent with expanded host range.

Keywords: baculovirus, biological control agent, common cutworm, lily cutworm, occlusion body

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INTRODUCTION

Insect viruses, specifically the baculoviruses, are among the most important biocontrol agents for biopesticide development. Baculoviruses comprise a large family of insect and other arthropod-specific viruses, the *Baculoviridae*, which is composed of four genera. These genera include the *Alphabaculovirus*, a lepidopteran-specific NPV; *Betabaculovirus*, a lepidopteran-specific granulovirus; *Gammabaculovirus*, a hymenopteran-specific NPV; and *Deltabaculovirus,* a dipteran-specific NPV (Jehle *et al.* 2006). Baculoviral diseases in insects have been recorded in over 85 species, wherein most of them target the lepidopterous larvae (ICTV 2019).

Viruses from family *Baculoviridae* such as alphabaculoviruses and betabaculoviruses are among the insect pathogens of the genus *Spodoptera* that can be used in microbial control, together with other control tactics (Rao *et al.* 2007). Alphabaculoviruses are enveloped rod-shaped virions carrying a circular dsDNA genome. It produces a proteinaceous crystal inclusion bodies, which contains a large number of progeny virus particles in the infected host cell nuclei (Granados and Williams 1986). In *Spodoptera litura* larva*,* NPV infection is commonly characterized by the pinkish and fragile integument and oral discharge of watery fluids (Paris 1969).

Baculoviruses may show both high host-specificity and intense virulence to its susceptible hosts. Moreover, virions are protected against inactivating agents by the presence of polyhedral OBs, which they can retain infectivity for long years (Groner 1987); thus, baculoviruses are considered as one of the most promising insect microbial control agents.

The NPV host range is reported to vary with the interaction of the virus with the insect host (Cheng and Lynn 2009; Wang *et al.* 2008). The host range and the cross-infectivity of many baculoviruses were reviewed by Groner (1987). The usual parameters of NPV pathogenicity are the presence of signs and symptoms of viral infection and mortality of the larvae after *per os* infection (Bishop *et al.* 1995).

In general, the host range of most NPV is restricted to one or a few species of the same genus or family of the host where it was originally isolated. NPVs are widely distributed among about 54 arthropod species belonging to three insect orders: Lepidoptera (51 species), Hymenoptera (two species), and Diptera (one species) (ICTV 2019). In spite of the great potential of NPV as a biocontrol agent of lepidopterous pests, it has been studied only in a limited extent here in the Philippines. This is because of some limitations such as narrow host range. However, there are few exceptions of NPV having a broader host range such as the *Autographa californica multiple nucleopolyhedrovirus* that infects more than 30 species from about 10 insect families, which all belong to the order Lepidoptera. *Anagrapha falcifera nucleopolyhedrovirus* has also a broad range in which it can infect more than 31 species of Lepidopterans from 10 families. *Mamestra brassicae multiple nucleopolyhedrovirus* is also known to infect 32 out of 66 tested Lepidopteran species belonging from four different families (Doyle *et al.* 1990; Hostetter and Puttler 1991).

Lily cutworm, *Spodoptera picta*, is a newly reported species in the Philippines (Lit *et al.* 2014). The larva is a voracious feeder of lily plants (Liliaceae), particularly *Crinum* species. They pose damage by feeding on the leaf surfaces and leaf sheath at the base of the plant. It can cause severe damage to lilies, which often kill the plant (Ang *et al.* 2010). On the other hand, the common cutworm, *S. litura* is a more important insect pest in the country from the same genus. It is a polyphagous insect pest that feeds on almost all kinds of green vegetation (Paris 1969). It is reported to infest agricultural crops such as rice, corn, onion, banana, peanut, and cotton (Gabriel 1997).

S. litura is naturally susceptible to NPVs. Laviña *et al.* (2001) biologically characterized *Spodoptera litura nucleopolyhedrovirus* (SpltNPV) isolated from the Philippines. The SpltNPV shows great potential as a biocontrol agent and was developed to control *S. litura* on vegetables, cotton, rice, and peanuts in China, India, and Taiwan (Moscardi 1999). In the Philippines, farmers used SpltNPV on eggplants heavily damaged with *S. litura* and observed significant decrease of cutworm population in the field (Navasero and Navasero 2003).

This paper first reports an NPV isolated from *S. picta*, SppiNPV. In addition, the cross-infectivity of SppiNPV to *S. litura* was evaluated since SppiNPV could offer an option for the management of the common cutworm, particularly against field strains resistant to *S. litura* NPV infection (Fuxa and Richter 1990). This study, therefore, aimed to determine the cross-infectivity of the NPV isolated from *S. picta* against *S. litura* larvae. The SppiNPV OBs, as well as the sign and symptoms of SppiNPV infection and its pathogenicity on *S. litura*, were also described.

MATERIALS AND METHODS

S. picta and NPV Source

Egg masses and larvae of *S. picta* were collected from giant crinum lily (*Crinum asciaticum* L.) plants in Barangay Dayap, Calauan, Laguna. Larvae were brought in the Insect Physiology Laboratory at IWEP, CAFS, UPLB and maintained at 27 ± 1 °C at 70 ± 10% relative

humidity with photoperiod of 12:12 (light: day). Fresh and clean yellow lily leaves were provided as necessary. Fully-grown larvae that were about to pupate were transferred into a rearing tray with sterilized moist soil as a pupation medium. After 4–5 d, the pupae were collected and transferred into emergence cages measuring 43 x 30 x 30 cm. The emergence cage was provided with a 10% honey solution as food for the emerging adults, and fresh yellow lily leaves to serve as oviposition medium.

After three generations of rearing *S. picta,* larvae exhibiting NPV signs and symptoms were observed. These unhealthy larvae were collected and separated from the rearing for NPV extraction. The signs and symptoms of the NPV-infected *S. picta* larvae were observed and recorded.

Collection of Test Host Insect – *S. litura*

Egg masses of *S. litura* were collected in the field either in corn, banana, taro, or legumes. Newly-hatched larvae were reared in the laboratory using castor leaves. Freshly harvested and clean castor leaves were provided as necessary. Likewise, larvae were transferred into freshly cleaned containers as needed until it reached its third instar larval stage. These field-collected larvae were used for the cross-infectivity evaluation of SppiNPV.

Preparation of SppiNPV Inoculum

A stock SppiNPV OB suspension was prepared from *S. picta* larvae. Dead larvae showing NPV infection were pooled into a sterile Erlenmeyer flask and were then triturated using a glass tissue homogenizer. The resulting homogenate was filtered through two layers of sterile muslin cloth, and the filtrate was centrifuged at 11,600 x g at 4 °C for a minute to partially purify the OBs. The OBs were washed with 750 µL sterile distilled water. The centrifugation and washing were done thrice but on the third time, OBs were washed in 500 µL distilled water and were resuspended in 1 mL sterile distilled water. This OB suspension was used as a stock solution in the succeeding experiments. The OBs of SppiNPV were observed and described.

SppiNPV Infection in *S. litura* and Determination of Larval Weight Gain and Mortality

Third instar larvae of *S. litura* were used in this study. Each larva was individually placed in a transparent cylindrical container with a diameter of 5 cm covered with a transparent lid. Each larva was fed with 100 OBs by overlaying 10 µL of 10^4 OBs/mL solution onto an artificial diet cube (~ 1.5 cm^3). The control group, on the other hand, was fed with 10 µL sterile distilled water overlaid onto an artificial diet cube. After 24 h, the unconsumed treated artificial diets were removed and the untreated artificial diets were continually given as food.

Each larva was then weighed at 2, 4, and 6 dpi. Only the larval weight gain at 4 dpi relative to larval weight at 2 dpi was calculated for each larva as a parameter to check for normal development since larva to pupa transformation already started at 6 dpi. Mortality was recorded daily until insects died at the larval and pupal stages. Those individuals that survived until adulthood were also observed for abnormalities. Thirty (30) biological replications were used for each of the control and NPV-infected larvae.

Data on larval weight and weight gain were analyzed using two-sample t-tests, and one-way analysis of variance with Tukey's *post hoc* test. Differences were considered significant for $P < 0.05$.

Examination of the Diseased *S. litura* Larvae

Infected larvae were examined under a dissecting microscope and characteristic external and internal signs and symptoms of NPV-infection were noted. Thin films of hemolymph from the NPV-infected larvae were also examined under the oil immersion objective (1,000x magnification) and were compared with the hemolymph from the healthy larvae. The OBs present in the infected NPV larvae were observed and described. Signs and symptoms of SppiNPV infected *S. litura* were observed and recorded.

RESULTS AND DISCUSSION

Observation of SppiNPV Infection in *S. picta* Rearing

An NPV-like disease was observed in a laboratory colony of *S. picta*, established from the field population, after three generations. This indicated that a covert baculovirus infection occurred in *S. picta,* thus – following the nomenclature of naming insect viruses by the International Committee of the Taxonomy of Viruses (ICTV) – the newly isolated pathogen was tentatively named *Spodoptera picta nucleopolyhedrovirus* (SppiNPV).

Williams *et al.* (2017) noted that covert infections, which could occur in both natural and laboratory populations of insects, are characterized by the absence of visible signs of the disease and is synonymous to inapparent, silent, or occult infections. In this type of infection, the pathogen is maintained in the natural population through vertical transmission of genotypic variants that are less pathogenic or less virulent than horizontally-transmitted genotypic variants (Cabodevilla *et al.* 2011). Burden *et al.* (2006) observed that these vertically-transmitted genotypic variants, although remains fully competent within their host, can be triggered to produce lethal disease. Although the mechanism of the maintenance of these vertically-transmitted genotypic variant baculoviruses in their host is

still unknown, it was hypothesized by Kane and Golovkina (2010) that possible strategies to maintain viral covert infection include the selection of specific cell types to maintain viral genomes, viral gene expression modulation, and avoidance of clearance by the host immune system.

Several studies have shown that the activation of baculovirus infection in apparently healthy insects is caused by physiological stress (Podgawaite and Mazzone 1986), brought about by overcrowding (Fuxa *et al.* 1999; Opoku-Debrah *et al.* 2013), an abrupt change in temperature and relative humidity (Fuxa *et al.* 1999; Kouassi *et al.* 2009), changes in nutrient availability (David and Gardiner 1966), and dehydration (Biever and Wilkinson 1978). The *S. picta* in this study has been exposed to such conditions; for instance, the field-collected larvae were reared in trays and supplied with fresh leaves and not the live host plants as food.

OBs of SppiNPV

Most NPVs are isolated from the orders Lepidoptera, Hymenoptera, and Diptera (Rohrmann 1986). The NPV development in lepidopteran larvae is most pronounced in the nuclei of the fat body, hemocytes, and trachea. In the advanced stages of disease development, the infected larva actually appears to be sluggish and discolored with an oily appearance. At the time of death, the oily cuticle becomes darker that contained liquefied internal tissues. After death, the cuticle becomes fragile and often ruptures to millions of polyhedral, which can contaminate the food of the host insect (Granados and Williams 1986).

The NPV can be observed in dead or dying larvae as bright irregular crystals called OBs (Grzywacz *et al.* 2004). These OBs – commonly called polyhedra – may exist as dodecahedra, tetrahedral, cubes, or irregular angular forms and many others contain several hundred virus particles. The diameter of the polyhedral viruses is large, which varies from 0.13–15 µm depending on a particular NPV (Kumar *et al.* 2011). SppiNPV produced rod-shaped OBs (Figure 1), a characteristic of alphabaculoviruses (Harrison and Hoover 2012).

The OBs are the infective stage of NPV and are responsible for the insect-to-insect transmission of baculoviral disease (Rohrmann 2019). The virions within the OBs are protected by the polyhedrin crystal from possible inactivation by various environmental factors. Baculoviruses in this form are extremely stable and can retain infectivity for many years, if not exposed to UV light or high temperatures (Williams *et al.* 2017).

Following consumption of OBs by the host insect, the polyhedral coat is digested; thus, occlusion-derived virions were liberated in the midgut (Rohrmann 2019). OBs are dissolved in the midgut lumen after ingestion,

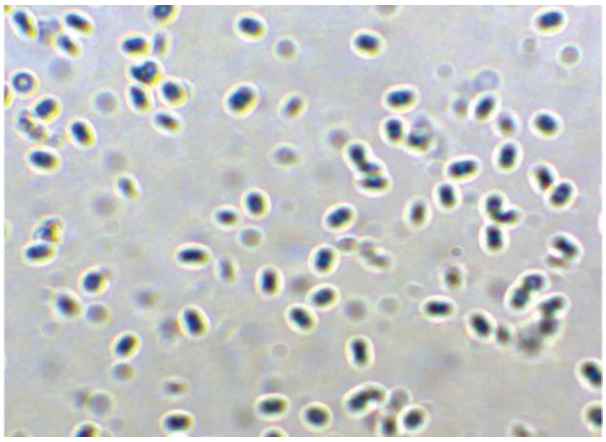

Figure 1. OBs of SppiNPV viewed under the microscope (1,000x).

releasing the embedded occlusion derived virus that infects epithelial midgut cells. The progeny budded virus buds from the epithelium basal surface, crosses the basal lamina, and infects the larva's remaining tissues (Clem and Passarelli 2013). The principal steps of the baculovirus infection cycle involve entry into the midgut cells, expression of viral genes, DNA replication, late and very late gene expressions, production and release of the budded virus, and OB formation (Slack and Arif 2007). The host range of baculoviruses is determined by its capacity to enter the insect host cells and tissues, replicate, and release new infectious virus particles (Thiem 1997).

Signs and Symptoms of SppiNPV Infection in *S. picta*

The integument, including the legs and head capsule of mildly-infected larvae, became light yellow. Abnormalities in the abdomen of the infected larvae were also observed. For instance, the seventh abdominal segment between the two thick black stripes appeared too bloated and color yellow. The 8–10 abdominal segments seemed to fuse and form a thick transverse black stripe that surrounded the body of the larva (Figure 2b). The fusion of the abdominal segments of the SppiNPV-infected larvae may be due to the viral manipulation of the larval metamorphosis (O'Reilly 1995; Ikeda *et al.* 2015). Some NPVs produce ecdysteroid uridine 5'-diphospho--glucosyltransferase (UGT), an enzyme encoded by the *egt* gene that affects the course of infection in their hosts (O'Reilly and Miller 1989). The viral ecdysteroid UGT inactivates the insect molting hormones (O'Reilly and Miller 1990) by catalyzing the transfer of glucose from uridine 5'-diphospho-glucose to ecdysteroids in NPV-infected insects, thus blocking molting and pupation. NPV-infected insects suffer severe physiological stress during molting, which usually caused death to the insects, and thus the action of the ecdysteroid UGT gives the NPV an evolutionary advantage by preventing this stress,

thereby allowing the full productivity of the virus infection (O'Reilly and Miller 1991).

A healthy *S. picta* is characterized by the presence of black stripe only on the first abdominal segment and just a pair of two dots on the 9th and 10th abdominal segment; orange prolegs on the 3rd, 4th, 5th, 6th, and 10th (last) abdominal segment; and an orange head capsule (Sevastopulo 1939), which was not observed on the infected larvae (Figure 2). Meanwhile, the mouthpart of NPV-infected *S. picta* larva was yellow instead of orange (Figure 2c). The mildly-infected larvae were also observed to be restless and mobile. These can be regarded as initial signs and symptoms of NPV infection in *S. picta*. At the advanced stage of infection, the *S. picta* became immobile and usually stayed at the bottom of the rearing container near the paper lining. The larvae were pink, became extremely fragile, easily lysed, and ruptured (Figure 2d).

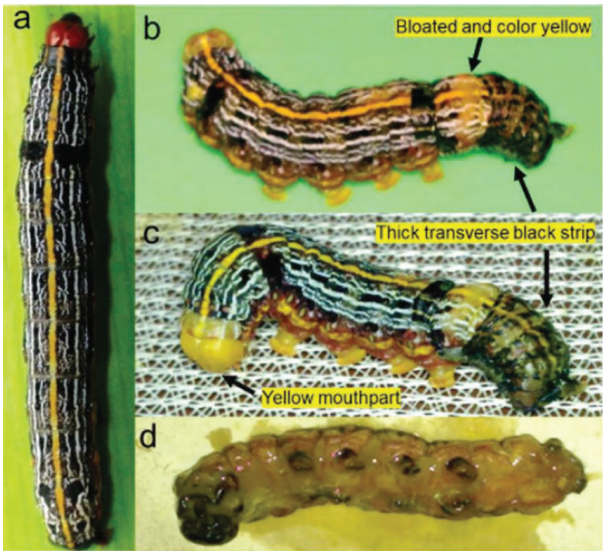

Figure 2. *Spodoptera picta* larvae: (a) healthy (Ang *et al.* 2010) and infected with SppiNPV showing (b–c) the bloated seventh abdominal segment, yellowing of the integument in between of two black strips (with 8th to 10th abdominal segment fused to develop a black strip), and presence of yellow mouthpart instead of orange; and (d) lysed and ruptured SppiNPV-killed *S. picta* with liquefied internal organs.

Effect of SppiNPV Infection to *S. litura* Weight Gain

At 2 dpi, cutworms were already in their fourth larval instar while at 6 dpi, they were already in the fifth larval instar. The fast larval growth and molting may be due to the artificial diet, which contained more nutrients than that of the host plants, provided to the control and treated larvae. At 6 dpi, cutworms were either on their last larval instar or already in the pre-pupal stage; thus, lower weight gain was observed at 6 dpi than at 4 dpi. Generally, heavier larvae were recorded in the untreated cutworms than the NPV-treated cutworms; however, a significant difference in mean weight was only observed at 4 dpi. However, in terms of average weight gain, the control group (0.342 ± 0.028 g) did not significantly differ from the SppiNPV-treated larvae (0.273 ± 0.027 g) (Table 1). The results suggest that SppiNPV was not virulent enough to *S. litura* to affect weight gain.

NPV infection usually results to greater larval weight gain since they feed more to grow more tissues for OB production (O'Reilly and Miller 1991), thus releasing more OBs for an increased chance of the virus to infect more insect hosts (Carballo *et al.* 2017). The positive correlation between larval weight gain and OB production has been reported by Cory and Myers (2003). In contrast, Ali *et al.* (2019) reported that NPV impaired food consumption and reduced weight gain relative to the untreated *S. litura* larvae, which was observed in this study.

In the *in vivo* mass propagation of NPV, continuous growth and development of larvae up to its maximum size is desired because this means that there is the production of polyhedral inclusion bodies and continuous propagation of the virus inside the host body since more cells are available as infection sites. If there is early death of the host, this would result to lower NPV yield (Ebora 1987).

Pathogenicity of SppiNPV to *S. litura*

The mortality of third instar *S. litura* fed with SppiNPV is presented in Table 2. At 4 dpi, an *S. litura* larva died in SppiNPV-treated set-up, another larva died at 5 dpi, and four at 6 dpi – thus resulting to a cumulative mortality of 20%, which is significantly different from the untreated larvae (0%). The sublethal effect of SppiNPV-infection in *S. litura* larvae was observed until 9 dpi. From 7–9 dpi, six larvae did not complete the larval-pupal transformation and eventually died, thus resulting in significant

Table 1. Mean weight and weight gain (g) of *Spodoptera litura* at different dpi infected with 100 OBs per larva of SppiNPV[a].

Treatments	Mean weight[1]			Weight gain at 4 dpi[ns]
	2 dpi[ns]	4 dpi*	6 dpi[ns]	
SppiNPV	0.354 ± 0.016	0.627 ± 0.031[b]	0.344 ± 0.017	0.273 ± 0.027
Control	0.382 ± 0.011	0.724 ± 0.029[a]	0.387 ± 0.016	0.342 ± 0.028

[a]Means followed by the same letter(s) within the same column are not significantly different at 5% level of Tukey's HSD test; n = 30

Table 2. Mortality of *Spodoptera litura* infected, as third instar, with 100 OBs of SppiNPV per larva at different dpi (n = 30)[a].

Treatments	Mortality count at specific dpi									% mortality		
	Larval stage					Pre/pupal stage				Larval stage**	Pupal stage**	Total**
	1	2	3	4	5	6	7	8	9			
SppiNPV	0	0	0	1	1	4	1	2	5	20[a]	26.67[a]	46.67[a]
Control	0	0	0	0	0	0	0	0	0	0[b]	0[b]	0[b]

[a]Means followed by the same letter(s) within the same column are not significantly different at 5% level of Tukey's HSD test; n = 30

cumulative mortality of 47.67%, while still no mortality was recorded in the control group at 9 dpi (Table 2).

Based on the observed mortality, the concentration of OBs used to infect the larvae may not be enough to cause higher percentage mortality to the test cutworm larvae population. The pathway of NPV infections has been summarized by Tinsley (1979) and Granados and Williams (1986). Ingestion of polyhedra until the death of larva usually takes about 4 d to 3 wk upon infection depending on the different NPVs, insect hosts, number of polyhedra ingested, and environmental temperature. Nonetheless, the results suggest that SppiNPV can cross-infect and was pathogenic to *S. litura* but was less virulent at the 100 OBs per larva. Ali *et al.* (2019) performed leaf disk bioassay of fourth instar larvae of *S. litura* infected with SpltNPV at two doses: 10^4 and 10^5 OBs per larva. About 33.33% and 80% mortalities were observed at 10^4 and 10^5 OBs per larva, respectively. Meanwhile, Barreto *et al.* (2005) confirmed that all the *Spodoptera frugiperda multiple nucleopolyhedrovirus* isolates they tested to *S. frugiperda,* showedhighmortality (> 72%) when using concentrations equal or more than 10^6 OBs/mL. In addition, the observed low virulence of SppiNPV to *S. litura* may also be due to the presence of the less-virulent genotypes since the OBs were initially isolated from covertly-infected *S. picta* field population.

SppiNPV-infected Hemolymph of *S. litura*

The hemolymph of SppiNPV-infected *S. litura* larva was examined under a microscope and was compared with the hemolymph of a healthy larva. As expected, OBs were observed in the hemolymph from the larva infected with SppiNPV (Figure 3). In addition, no hemocytes were observed in the diseased larvae since all the hemocytes were infected with the polyhedral inclusion bodies, confirming productive SppiNPV infection in *S. litura.* The presence of OBs also suggests the cross-infectivity of SppiNPV to *S. litura.* There are many reports of cross-infectivity of NPV using oral routes of infection and high doses of virus inocula (Aizawa 1963; Harper 1973; McKinley *et al.* 1981). Harpaz and Raccah (1978) confirmed that *S. littoralis* NPV can cross-infect the larvae of *S. exigua* and *S litura* but failed to cross-infect other lepidopterous larvae from another genus.

Signs and Symptoms of SppiNPV in *S. litura*

SppiNPV infection at fourth and fifth instar larvae was characterized by the pinkish coloration of the cuticle, a characteristic sign in *Spodoptera* species. The integument of the mildly-infected larvae was easily twisted when touched and expelled slimy fluid from their mouth. Infected larva exhibited pinkish or brownish cuticle, while the healthy larva possessed black integument (Figure 4). At the advanced stage of infection, the larvae became

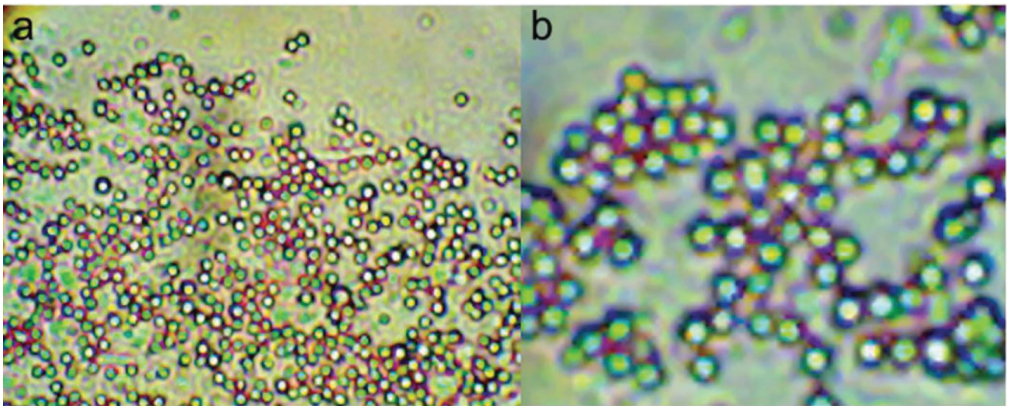

Figure 3. OBs of SppiNPV isolated from *Spodoptera litura* viewed under a compound microscope at (a) 400x and (b) 1,000x magnification.

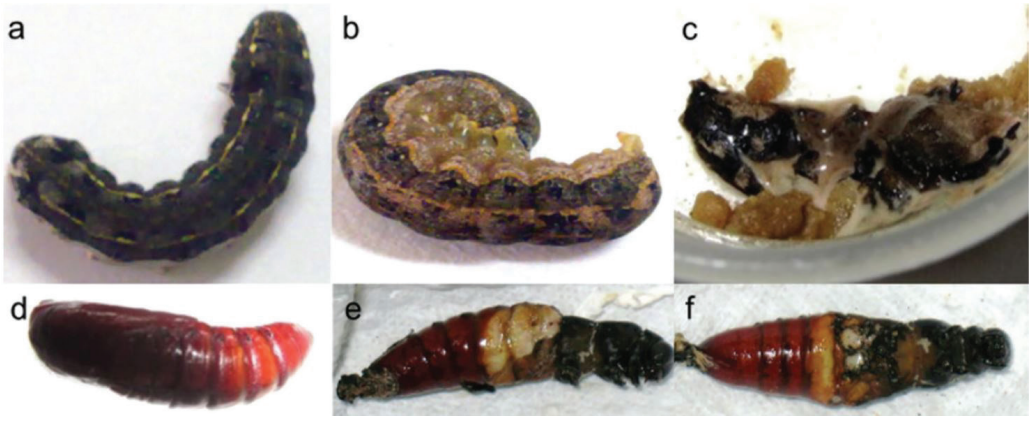

Figure 4. Signs and symptoms of SppiNPV infection in *Spodoptera litura*: healthy (a) larva and (d) pupa were shown as references; (b) *S. litura* with mild SppiNPV infection; (c) SppiNPV-infected larva showing liquefied internal organs; and (e–f) larva-pupa intermediates resulted from SppiNPV infection.

extremely fragile, easily lysed, and ruptured (Figure 4c). The fragility and lysis are due to the complete destruction of the epidermal cells, which is a common sign of NPV infection in *S. litura* larvae (Ebora 1987).

The symptoms observed in this study were also the same as reported by Barreto *et al.* (2005), wherein pink internal organs of NPV infected *S. frugiperda* were noted, totally destroyed, and liquefied at the time of death. Valicente (1988) stated that discoloration, paleness, integument liquefaction, and loss of appetite are common symptoms of baculovirus-infected larvae. Liquefaction of infected cadavers is known to enhance horizontal transmission of NPVs since it facilitates the release of OBs to the environment for dispersal (Hawtin *et al.* 1997). A successful infection usually depends on the ingestion of sufficient virus to initiate replication in the host. The uptake of the virus is regulated by larval behavior, feeding rate, and viral concentration and distribution (Hunter *et al.* 1984).

Baculoviruses are very safe to humans, and their prominent feature as an insect pest biocontrol agent comes from their characteristic ability to generate sequestered virions within the crystalline protein matrix of OBs (Rohrmann 1992). The OB enables the survival and dispersal of viruses in the environment and can be turned into a convenient, safe, and easily manipulated product (Bulach *et al.* 1999).

NPVs into the susceptible insect cells may produce enzymes that help liquefy the tissues and the cuticle, thus ensuring the survival of SppiNPV by an increased chance of being ingested by a susceptible host. Chitinase may not be required in the initial stages of NPV infection *in vivo*, but it has important roles in host liquefaction (Daimon *et al.* 2007) and assists in permeabilizing the peritrophic matrix (Hawtin *et al.* 1995). Baculovirus cathepsin is transcribed at later times from about 9–12 h post-infection (Hodgson *et al.* 2007) and, together with chitinase, is critical in promoting liquefaction of the host cadaver at its final stage (Slack *et al.* 1995). In addition, cathepsin is also required for melanization of the host, which occurs near insect death (Slack *et al.* 1995).

Morphological abnormalities were also observed in the infected individuals. Although the mortality in SppiNPV-infected *S. litura* larvae was low at 9 dpi, five SppiNPV-infected larvae failed to complete pupation resulting in larval-pupal intermediates (Figures 4e–f). At 16 dpi, all the cutworm larvae in the control set-up developed as normal pupae while three surviving pupae in the SppiNPV-treated group, exhibited whitish-yellow tissue, and emerged as

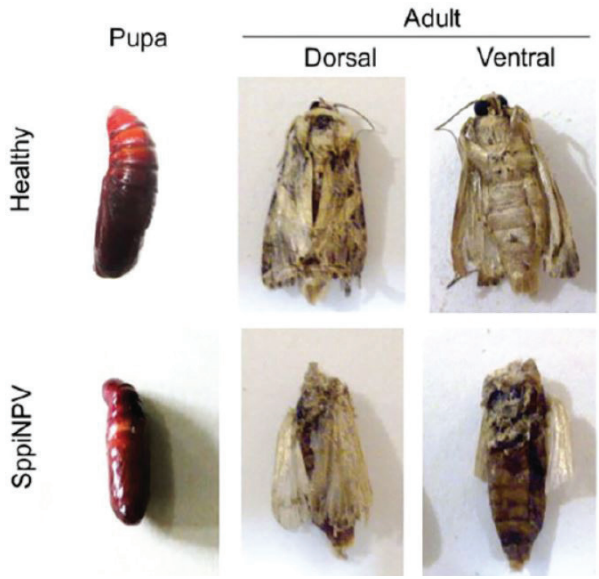

Figure 5. Malformations in the pupa and adults of *Spodoptera litura* infected with SppiNPV as larvae. Pupae and adults were photographed at 9 dpi and 16 dpi, respectively.

adults with short malformed wings (Figure 5). Moreover, the SppiNPV-infected adults displayed a brown abdomen that was soft, fragile, and easily lysed (Figure 5).

Previous studies by Legacion and Gabriel (1978) and Padma Vathamma and Veeresh (1989) noted that larva surviving NPV-infection may develop as adults but with short and malformed wings. Moreover, adults of *S. litura* NPV-infected larvae may still appear to have normal wings, emerge apparently as normal adults capable of reproduction, but may produce no progeny (Ebora 1987). The same was observed in SppiNPV-infected cutworm larvae in this study, where sublethal NPV-infection still resulted to pupal formation and adult emergence but with obvious malformations.

CONCLUSION

A putative NPV infective for *S. picta* was isolated for the first time and was tentatively assigned the name *Spodoptera picta nucleopolyhedrovirus* or SppiNPV. The SppiNPV produce characteristic signs and symptoms of baculoviral infection in both *S. picta* and *S. litura* larvae, indicating SppiNPV cross-infected the latter. However, the larval weight in the SppiNPV infected individuals was not significantly different from the control group. Some surviving larvae beyond 6 dpi either failed to pupate or emerged as malformed adults with an abnormally fragile abdomen, indicating the potential of SppiNPV as a biological control agent of *S. litura* even at sublethal doses. Thus, further studies are needed to further characterize this novel SppiNPV, increase its virulence through a continuous passage in *S. litura* and *S. picta*, and determine its host range. Moreover, optimization of the amount of OB to ensure 100% mortality is needed for formulation and field application purposes.

ACKNOWLEDGMENTS

We thank the Department of Science and Technology – Science Education Institute – Accelerated Science and Technology Human Resource Development Program for the scholarship awarded to Ms. Abigaile Mia V. Javier-Hila. We also thank Dr. Pio A. Javier for providing the initial colonies of the test insects and the laboratory to perform the rearing.

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