A Nuclear-Polyhedrosis Virus of *Spodoptera frugiperda* Isolated in Puerto Rico[1,2]

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ABSTRACT

A nuclear-polyhedrosis virus was isolated from a larva of *Spodoptera frugiperda* collected in Puerto Rico. The virus was found to be pathogenic to the larvae of *Heliothis zea* and of *S. frugiperda* but nonpathogenic to those of *Diatraea saccharalis* and of *Galleria mellonella*. LD_{50} for the fourth instar larvae of *H. zea* and *S. frugiperda* inoculated *per os* were 1.25×10^3 and 2.7×10^3 polyhedral inclusion bodies, respectively. The tissues infected included hemocytes, fat body, muscle, and epidermis. Furthermore, transovarian transmission of the virus was found in the inoculated individuals of *S. frugiperda*.

INTRODUCTION

Nuclear-polyhedroses, viral diseases caused by members of baculoviruses, have been recognized in a Lepidopterous insect pest, *Spodoptera frugiperda* (1, 4). Because of their usefulness in controlling insect pests, some of those viruses have been considered as potential viral insecticides (7, 9).

During a survey of microorganisms associated with the insects of Puerto Rico, a nuclear-polyhedrosis virus was isolated from a dead *S. frugiperda* larva in Isabela. Because of its potentiality as a microbial pest control agent, the author conducted a series of experiments to elucidate the nature of the virus and of its infection. This article describes morphological characters, virulence, host range, and transovarian transmission of the virus, and histopathology of the viral infection.

MATERIALS AND METHODS

REARING OF INSECTS AND PROPAGATION OF THE VIRUS

Heliothis zea, Spodoptera frugiperda, and *Diatraea saccharalis* used in the experiments were individually reared in plastic cups containing an artificial diet developed previously (10). *Galleria mellonella* were reared in large bottles with a diet consisting of a "Mixed" cereal powder (Gerber Products Co., Fremont, Michigan)[4] (208 g), glycerol (66 ml), and pure

honey (134 ml) per 200 ml medium. Laboratory colonies of the insects, as well as virus-treated larvae, were reared in two separate incubators at 28°C without light.

For the virus propagation, the original virus-infected larva was homogenized and the homogenate filtered through double layers of cheesecloth. The filtrate thus obtained (crude polyhedra suspension) served as a source of primary inocula for further propagation. Larvae of *S. frugiperda* (third through fifth instar) were starved for half a day and fed small amounts of the artificial diet contaminated with the crude polyhedra suspension. Those killed by the virosis were collected, homogenized, and the homogenate was filtered as above. The polyhedra fraction served as a source of the experimental inocula.

MORPHOLOGICAL CHARACTERS OF THE VIRUS

Size and shape of the viral polyhedral inclusion bodies (PIBs) were studied in wet mounts with a phase-contrast microscope as well as with
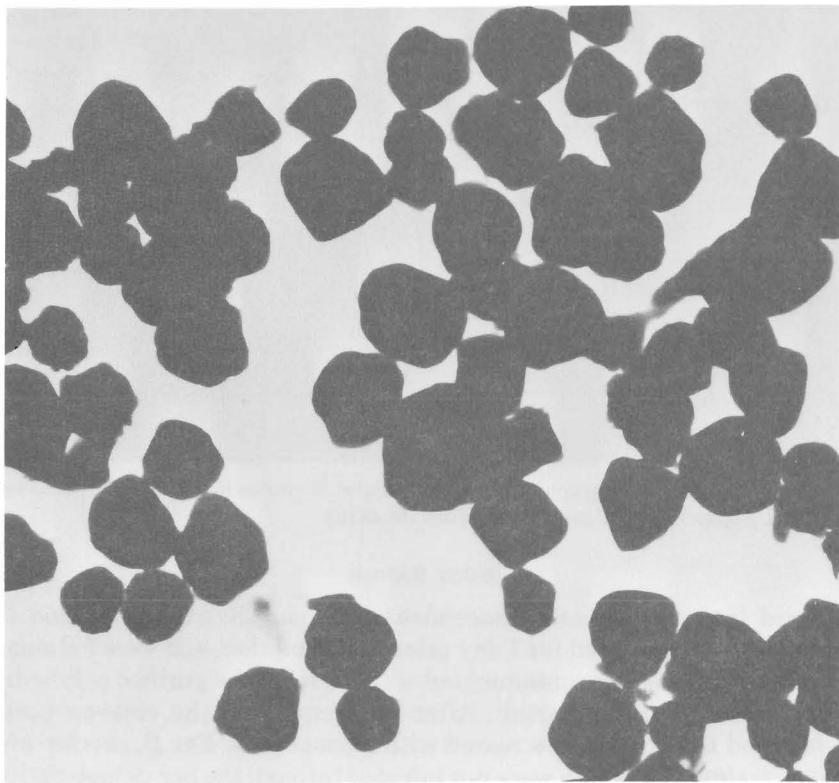

Fig. 1.—Electron micrograph of the polyhedral inclusion bodies of *S. frugiperda* NPV isolated in Puerto Rico (8,500×)

an electron microscope. For electron microscope observation the PIBs were fixed by Dr. Jean R. Adams of Beltsville, Maryland, first with 2.5% glutaraldehyde and then with 1% osmium tetroxide, embedded in Dow epoxy resin (No. 334),[4] and the plastics sectioned with an ultramicrotome. The thin sections of the plastics were examined with an electron microscope (Phillips, Model 301).[4]

FIG. 2.—Electron micrograph of the ultrastructure of virions in a polyhedral inclusion body of *S. frugiperda* NPV from Puerto Rico (58,438×)

HOST RANGE

Third instar larvae of *D. saccharalis, H. zea, S. frugiperda* and *G. mellonella* were starved for 1 day prior to inoculation, and were fed small pieces of artificial diet contaminated with 0.2 ml of the purified polyhedra suspension (4.7×10^8 PIBs/ml). After consumption of the virus-contaminated food the larvae were reared with normal food. For *D. saccharalis* and *G. mellonella,* which were not infected through the *per os* inoculation mentioned above, the author attempted intrahemocoelic injection of

alkali-liberated virions. The PIBs were dissolved in 0.03M Na_2CO_3 and 0.05M NaCl for 2.5 h and centrifuged, according to the method of Bergold (2). The pellet of liberated virions was dissolved in 1.0 ml of sterile distilled water, and aliquots of 2.4 microliter were injected intrahemocoelically into fourth instar larvae.

VIRULENCE

Larvae were anesthetized in carbon dioxide gas. Virulence was studied with *per os* inoculation of 100 anesthetized fourth instar larvae of *S. frugiperda* and *H. zea* with doses of 10^2, 10^3, 10^4, and 10^5 PIBs;[5] a syringe was attached to a microapplicator. The inoculated larvae were fed the artificial diet, and the mortality by the virosis was recorded.
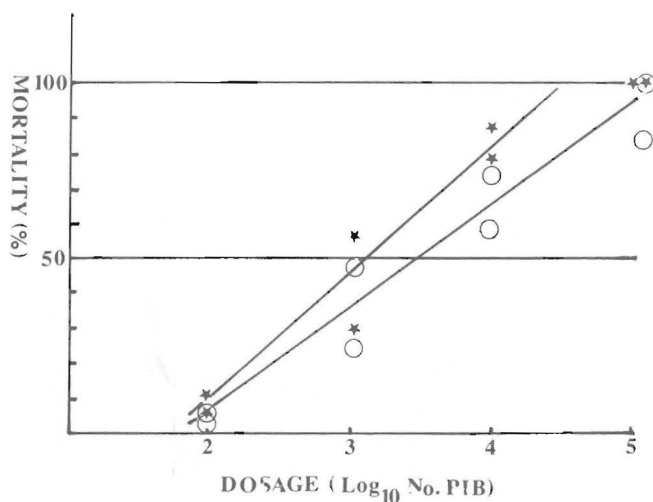

FIG. 3.—Dosage-mortality relationship of *S. frugiperda* NPV (Puerto Rico isolate) with the larvae of *H. zea* and of *S. frugiperda*. ★ Heliothis; ○ Spodoptera

HISTOPATHOLOGY OF VIRAL INFECTION

Third instar larvae of *S. frugiperda* were fed virus-contaminated artificial diet. Portions of the treated insects were collected at 6-h intervals and fixed in hot alcoholic Bouin solution. The tissues were dehydrated in a series of ethanol (50–100%), cleared in methanol, methylbenzoate, and benzene, and finally embedded in paraffin. The paraffin-embedded tissues were sectioned in thicknesses of 8 microns and stained with an azan stain according to Hamm (6).

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[5] After the study was concluded, it was found that a small portion of the PIBs was single-embedded, while the majority was multiply-embedded. Therefore, the inoculum was heterogeneous in composition.

TRANSOVARIAN TRANSMISSION

Fifth instar larvae of *S. frugiperda* were fed food contaminated with 0.5 ml each of purified polyhedra suspension (4.7×10^8 PIBs/ml). Adults that emerged from those larvae were allowed to mate. The egg masses obtained from 10 different females were surface-sterilized with 0.5% sodium hypochlorite solution for 20 min, rinsed in sterile water and incubated at 28 °C. The hatched larvae were reared with regular diet. Portions of the eggs, as well as all diseased larvae, were examined microscopically for the detection of PIBs. The sterilized water used for rinsing the eggs, previously treated with sodium hypochlorite solution, was smeared on top of the artificial diet, and healthy larvae (second and third instars) were fed the treated diet. That the larvae developed no virosis confirmed the efficacy of the surface sterilization.
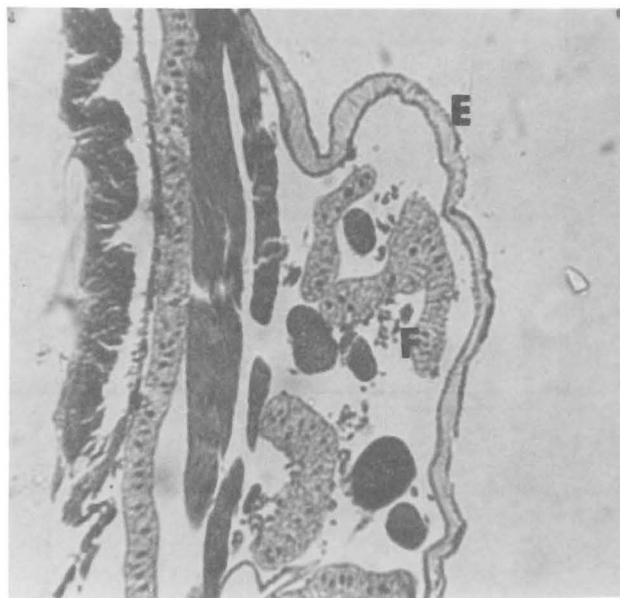

FIG. 4.—Section of a normal *S. frugiperda* larva. E: epidermis; F: fat body (400×)

RESULTS AND DISCUSSION

The results obtained in the present studies revealed irregular shape and size of PIBs in the Puerto Rico isolate of *S. frugiperda* NPV, as is evident in figure 1. The diameter of the longest axis of the PIBs ranged from 0.59 to 2.23 microns in wet mount. Thin sections of the PIBs demonstrated rod-shaped virions multiply-embedded and surrounded with at least two membranes (fig. 2), which resembled the NPV of *S.*

frugiperda studied by Bergold (3). In addition, a small fraction of the PIBs in the Puerto Rico isolate was found to be singly-embedded (or SEV). Since SEV was also previously reported from NPVs of *H. zea, S. frugiperda,* and *Trichoplusia ni* (5), heterologous composition of PIBs, in terms of the mode of occlusion of virions in viral polyhedra, appears to be common in the natural populations of NPVs affecting various species of Noctuidae.

The virus infected *H. zea* and *S. frugiperda,* both of which are Noctuidae. However, neither *per os* nor intrahemocoelic inoculation induced the virosis in *D. saccharalis* or in *G. mellonella*; this fact indicates a strong host specificity at the family level. Although host specificity is often used as one of the important criteria for the classification of baculoviruses, similarities in morphological characters and host
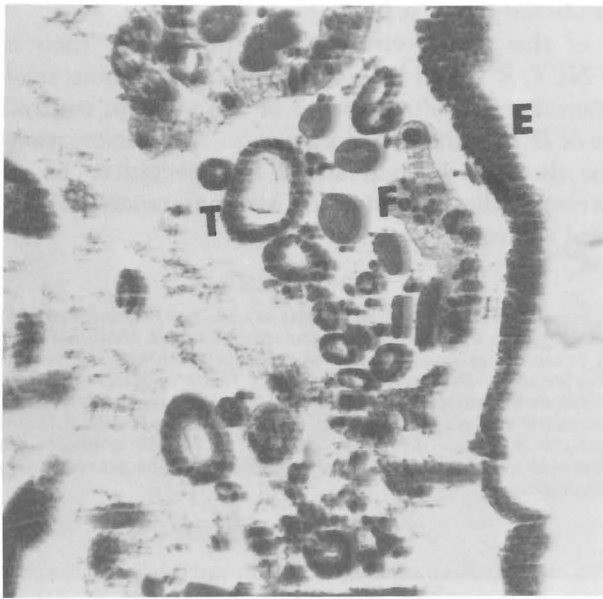

FIG. 5.—Section of an infected *S. frugiperda* larva. E: epidermis; F: fat body; T: trachea (400 ×)

range alone do not necessarily prove that the Puerto Rico isolate is identical to the NPV of *S. frugiperda* discovered elsewhere, because of a criticism on the conventional classification of baculoviruses (9). Furthermore, a recent DNA homology study of 4 NPVs isolated from 4 closely-related species of *Spodoptera* revealed a wide range of difference (8).

Figure 3 shows a dosage-mortality relationship of the viral infection in *S. frugiperda* inoculated *per os*. From the results obtained, LD_{50} for the

fourth instar larvae of *H. zea* and *S. frugiperda* were calculated as 1.25×10^3 and 2.70×10^3 PIBs, respectively. It is evident that *H. zea* is more susceptible to the virus than *S. frugiperda*.

In contrast to normal tissues (fig. 4), histological examination of the infected insects revealed that hemocytes, fat bodies, muscles, and epidermis were heavily infected with the virus, showing densely stained aggregates of PIBs in nuclei (fig. 5). Extensive destruction of fat bodies and epidermis was often detected within 2 days after inoculation. Hypertrophy of the infected nuclei, which is common in the infection by other NPVs, was also observed (fig. 5).

Although the examinations of the eggs laid by virus-inoculated females did not reveal any PIBs, 1.6% of the hatched larvae died of the nuclear polyhedrosis. Since it was reported that the transovarian transmission of polyhedrosis viruses was actually trans-ovum transmission, the present result contradicted the past finding.

Because of the strong virulence of the Puerto Rico isolate of *S. frugiperda* NPV, it would be worthwhile to undertake small scale field tests to determine the effectiveness of the virus in controlling natural populations of *H. zea* and of *S. frugiperda*. The transmission mechanism of the virus through infected adults is expected to be an additional favorable factor in the dissemination and maintenance of the viral agent in the natural environment.

RESUMEN

Un baculovirus (virus de núcleo-poliedrosis) se aisló por primera vez en Puerto Rico, de una larva de *Spodoptera frugiperda*. Se infectaron larvas de *Heliothis zea* así como de *S. frugiperda*. A pesar de las inoculaciones intrahemocilial y oral, no se encontró ninguna infección en las larvas de *Diatraea saccharalis* y *Galleria mellonella*, lo que indica una gran especificidad de los huéspedes. Varios tejidos se afectaron, entre ellos cuerpos grasos, hemocitos, músculo y epidermis. Se determinó que las dosis letales (LD_{50}) del virus en las larvas de *H. zea* y Je *S. frugiperda* fueron 1.25×10^3 y 2.70×10^3 poliedros, respectivamente. También se encontró que el virus puede ser transmitido de una generación a otra por medio de huevos contaminados.

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