PATHOGENICITY AND HISTOPATHOLOGICAL STUDIES ON THE NUCLEAR POLYHEDROSIS VIRUS OF THE FALL ARMYWORM, *Spodoptera frugiperda* (ABBOT & SMITH, 1797) (LEPIDOPTERA, NOCTUIDAE)

M.A. Garcia[1]
C.F.S. Andrade[1]
J. Lauritis[1]
M.E.M. Habib[1]

INTRODUCTION

The fall armyworm, *Spodoptera frugiperda*, is regarded as a serious pest of corn and several other gramineous plants in many New World countries. Early reports indicated the occurrence of viral diseases in *S. frugiperda* larvae (CHAPMAN & GLASER, 1915; ALLEN, 1921 and BERGOLD, 1943). Recently, ANDRADE **et alii** (1978) reported the natural occurrence of Nuclear Polyhedrosis Virus (NPV) in populations of this insect pest in corn fields, located in State of São Paulo. GARCIA (1979) studied the efficacy of some biotic factors which normally occur and control populations of this insect pest and stated that the NPV is a promising agent for integrated pest control programs. KUNO (1979) isolated a NPV from the same species in Puerto Rico, and evaluated its pathogenicity in three lepidopterous species. He also described some histological alterations in tissues of the fall armyworm. Analyses of the DNA and RNA, as

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[1] Instituto de Biologia, UNICAMP, Campinas, SP.

well as some serological, morphological and physical properties of NPV isolated from *S. frugiperda* larvae and other related species of the same genus have been reported (DOUGHERTY & FAUST, 1969; BUD & KELLY, 1977; HARRAP **et alii**, 1977). HAMM (1968) compared the histopathological effects caused by NPV and Granulosis virus in *S. frugiperda* larvae.

The present work was undertaken to determine the susceptibility of *S. frugiperda* larvae to its NPV, and to reveal some histological alterations resulting from the viral infection. Determination of a sequence of polyhedra formation was also one of the purposes of this study.

MATERIALS AND METHODS

The polyhedral inclusion bodies (PIBs) were isolated from late instars of *S. frugiperda* larvae and purified adopting the methods described by BERGOLD (1953), STEINHAUS (1963), HARRAP **et alii** (1977) and ANDRADE (1981).

usceptibility tests (using LDs and LTs criteria) were done by oral infection of the 4th instar larvae. Small pieces of fresh corn leaves were contaminated with purified virus suspensions (5 doses) and offered to the larvae. According to the quantity consumed of these contaminated leaves, the number of PIB/larva was calculated. The mortality was corrected using the HENDERSON & TILTON (1955) formula. Fifty larvae were used for each treatment.

For histopathological studies, thirty larvae were infected with approximately 10^6 PIBs for each, and sacrified at 18, 24 and 36 hours after infection. Tissues were immediately fixed with 4% gluteraldhyde in 0.15 M cacodylate (pH = 7.2) for two hours at 4ºC followed by post-fixation with 1.0% osmium tetroxide in the same

buffer for three hours at 4ºC. Specimens were dehydrated in a graded acetone series and embedded in Epon. Thin sections were cut with glass knives using a Porter Blum MT2 ultratome, stained with uranyl acetate and lead citrate and examined with a Zeiss EM9-S2 electron microscope. Thick sections (1-2 µm) for light microscopy were treated with 1% periodic acid and then stained with toluidine blue.

RESULTS AND DISCUSSION

Susceptibility

The 4th instar larvae of *S. frugiperda* infected with 4.5×10^4 and 4.5×10^5 PIB, suffered a significant kill 5 days after infection under laboratory conditions of 27ºC and 74% RH. On the other hand, larvae infected with lower doses of PIBs (4.5×10^2 and 4.5×10^3) exhibited a killing effect 8 days after infection (table I). From the data in table I, the mortality of larvae infected with 4.5×10^4 PIB was approximately two times higher than larvae infected with 4.5×10^3 PIB at 5, 8 and 12 days after infection. However, there were no significant differences observed between the mortality caused by 4.5×10^4 and 4.5×10^5 PIBs at 12 days after infection.

According to the calculated lethal doses (table II), to obtain 90% mortality within 5 days, the larva would need to ingest a relative high dose of ca. 2.5×10^6 PIBs. On the other hand, to obtain 50% mortality in 20 days, the larva would only need to ingest about 600 polyhedra. Within 8 to 12 days, the LD_{50} for the 4 th instar larvae was ca. 10^3 PIB / larva, which is simillar to the results obtained by KUNO (1979) for the same species and ALLEN & IGNOFFO (1969) for *Heliothis zea* larvae.

The results in figure I indicate that 4.5×10^4

TABLE I - Corrected mortality % 5, 8, 12 and 20 days after infection in 4th instar of *S. frugiperda* larvae.

PIBs/larva	Corrected mortality %			
	5 days	8 days	12 days	20 days
4.5×10^2	-	34.0	34.0	47.0
4.5×10^3	27.2	40.0	46.0	79.0
4.5×10^4	68.8	88.0	94.0	100.0
4.5 x 105	74.0	100.0	100.0	100.0

TABLE II - LD_{50}, LD_{70} and LD_{90} of NPV in *S. frugiperda* larvae 5, 8, 12 and 20 days after infection.

Dose	PIBs / larva			
	5 days	8 days	12 days	20 days
LD_{50}	2.30×10^4	3.28×10^3	2.48×10^3	5.85×10^2
LD_{70}	1.40×10^5	1.01×10^4	7.43×10^3	1.88×10^3
LD_{90}	2.48×10^6	1.21×10^5	3.70×10^4	9.23×10^3

PIB/larva was the most effective dose, causing a median lethal time of 4 days. Lower doses, however, were insufficient to provoke a significant death in *S. frugiperda* larvae within the 4 day period.

The susceptibility levels of *S. frugiperda* larvae let us to conclude that the NPV which occur naturally in corn fields, located in State of São Paulo, exhibits a high level of virulence for this insect species. Therefore, the NPV may prove to be a useful biological control agent for the fall armyworm in corn fields.

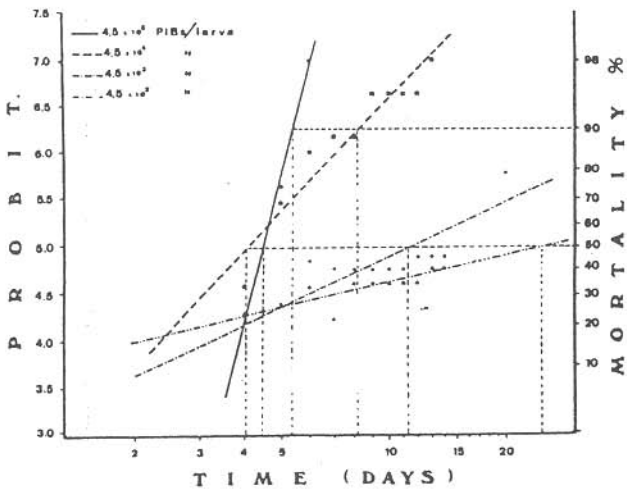

FIGURE 1 - Lethal times in *S. frugiperda* larvae after infection by 4 doses of NPV.

Pathology

S. frugiperda larvae exhibited the typical symptoms of baculovirus diseases. Slugishness in movements and loss of appetite were the first symptoms detected 18 hours after infection. Pallid coloration and initial flaccidity were observed 24 hours after infection, accompanied by some diarrhea and regurgitation. The larvae did not respond to mechanical stimuli 36 to 48 hours after infection.

During the final stage of disease, the larvae migrated to the top of the cages and died in a hanging position. Total flaccidity and integument fragility were observed immediately before death. The integument disrupted easily and turbid hemolymph flowed out. Death occurred within 2 to 6 days, according to dose, age and temperature.

During the course of the disease, the hemolymph showed a reduction in volume and total hemocyte count, lost its greenish shine coloration and showed a higher viscosity, indicating a possible desintegration of some tissues. The fat bodies were reduced in size and lost their healthy feature. Similar symptoms were reported in *Trichoplusia ni* (DRAKE & McEWEN, 1959) and *Anticarsia gemmatalis* (ALLEN & KNELL, 1977).

Polyhedra were initially observed in nuclei of some infected tissues, principally adipose, hypodermis and tracheal matrix. Some other tissues were also infected including muscle fibres and hemocytes. Fore-gut and hind-gut epithelial cells, Malpighian tubules, wing-buds, oenocytes and nervous ganglia were not invaded by the pathogen. This tissue involvement is not completely similar to those observed by SMITH (1967) and HAMM (1968).

It was not possible to establish a definite sequence of infection for the different tissues. However, adipose tissue was the first to be heavily infected (Fig. 2.A), and most of the nuclei were seen containing poly-

hedra. Nuclear and celular hypertrophy and chromatin clumping were the initial symptoms observed in the infected tissues. During the disease course, the number of PIBs increased reaching more than 100 per nucleus in fat cells (Fig. 2.C) and tracheal matrix (Fig. 2.B). In severely infected cells, many fibril bundles were seen in the cytoplasm (Fig. 2.D). Virus multiplication accompanied by the utilization of the genetic material have resulted in histochemical disturbances and citoplasmic breakdown. Nuclear disintegration and scattering of polyhedra in the cytoplasm were observed. Finally, break down of cell boundaries resulted in the flaccidity observed externally and the liquid content appeared full of polyhedra.

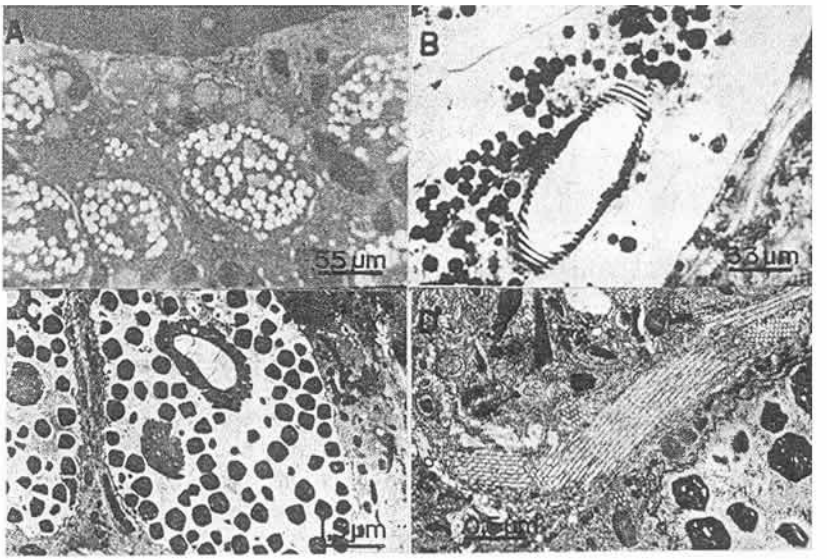

FIGURE 2- A. Macro-histopathological effects in fat tissue.
B. Polyhedra liberation in tracheal matrix.
C. Fat tissue nuclei full of polyhedra.
D. Fibril bundles in cytoplasm of an infected fat cell.

Our observations, concerning the formation of polyhedra, reveal that the arrangement of viral particles and bundles, in addition to their embedment in polyhedral matrix appear to be randomized. After replication, particles join to form bundles (3 - 14 particles each) with single membrane envelope (Fig. 3.A). These bundles appeared to be embedded in a partially clumped polyhedral matrix (Fig. 3.B). These matrixes, posteriorly polyhedra, continue to join viral bundles (Fig. 3, C and 3.D) until immediately before the mebrane formation of the polyhedra. Finally, completely formed polyhedra and scattered bundles as well as single rods appear within the infected nuclei (Fig. 3, E and 3.F).

RESUMO

O vírus da poliedrose nuclear, isolado de lagartas de *Spodoptera frugiperda*, revelou-se altamente patogênico para o 4º estádio larval do mesmo hospedeiro. Cerca de 10^4 poliedros por larva foram suficientes para causar 50% de mortalidade em 4 dias.

Os sintomas de infecção durante o desencadeamento da doença e os efeitos histopatológicos foram descritos. Possível sequência de formação de poliedros nos tecidos foi indicada.

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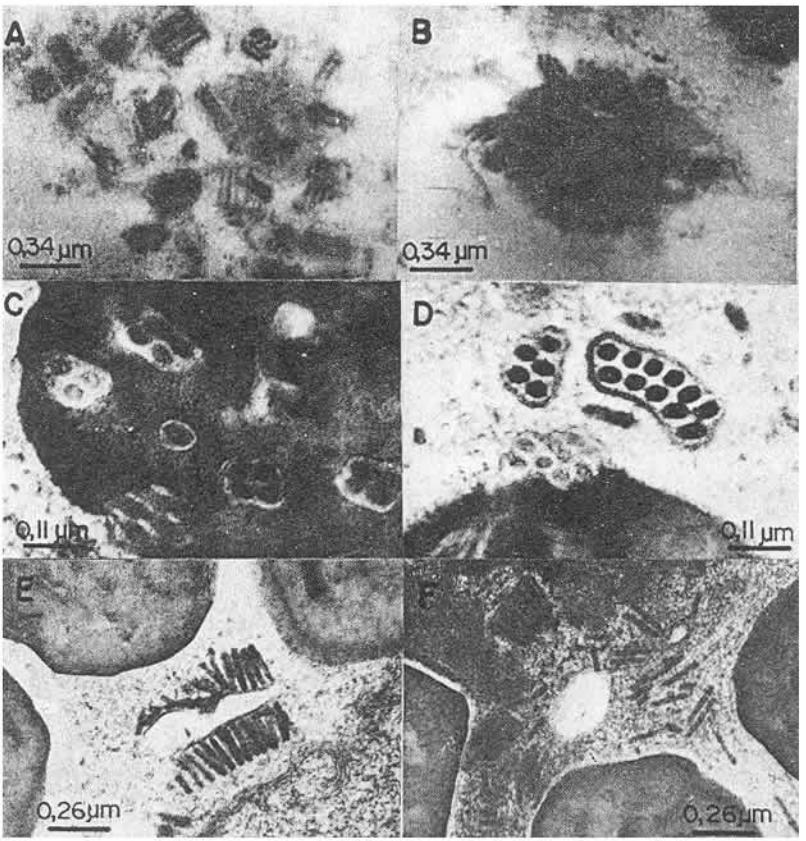

FIGURE 3. A. Randomized grouping of viral particles.
B. Embedment of viral bundles in protein matrix.
C.D. Advanced stages of polyhedra formation.
E.F. Viral bundles and single rods scattering in the nucleoplasm after polyhedra formation.

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