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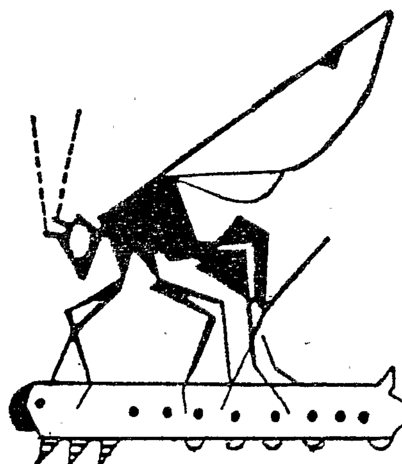

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HOST RANGE OF THE *AUTOGRAPHA CALIFORNICA* MULTICAPSID NUCLEAR POLYHEDROSIS VIRUS (ACMNPV) IN RELATION TO GRANULOSIS VIRUSES SUSCEPTIBLE LEPIDOPTERAN SPECIES IN EGYPT

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ABSTRACT

The host range of*Autographa californica* multicapsid nuclear polyhedrosis virus (AcMNPV) was studied in Egypt on 3 pest species susceptible to granulosis viruses. Among the cotton leafworm, *Spodoptera littoralis*, the maize corn borer, *Sesamia cretica* and the potato tuber moth *Phthorimaea operculella*, only *S. cretica* and *P. operculella* were susceptible to the AcMNPV. Ultrastructural studies and virus detection by nucleic probe showed that *S. cretica* was highly susceptible, while only 5% infection was obtained in *P. operculella.*

Key Words: AcMNPV, host range, GV, *Spodoptera littoralis*, *Sesamia cretica*, *Phthorimaea operculella.*

INTRODUCTION

The *Autographa californica* multicapsid polyhedrosis virus (AcMNPV) became a reference model for the viral genetic researches, biotechnology and biological control of insects. Its wide range of interest was due to several reasons. A large number of lepidopteran species belonging to twelve families, often representing important pests with high geographical distribution, were susceptible to AcMNPV (Albrecht, 1986). The AcMNPV could be mass produced and several of its hosts could be reared industrially on artificial diets (Vail *et al.*, 1972). The AcMNPV could be multiplied *in vitro* on a certain number of insect cell lines with large scale of origin (Granados and Hashimoto, 1989). The AcMNPV could be used as expression vector for broad range of genes with different origins (Eldridge *et al.*, 1992) and its great capacity of recombination could be used to modify its pathogenicity or host range by introducing foreign genes (Miller *et al.*, 1983).

As in our laboratory, the use of granulosis viruses (GVs) as biological control agents and as models for molecular studies represented a great importance, AcMNPV affinity of certain hosts which are susceptible to GVs in Egypt was examined. (Abol-Ela *et al.*, 1994;, Matthiessen *et al.*, 1978; Fédière *et al.*, 1992). The objective of this study is to obtain models which will permit the analysis of viruses produced from genetic recombinations between GVs and NPVs.

MATERIALS AND METHODS

Virus

AcMNPV strain was obtained from Dr. Croizier (INRA, France). For conserving the initial viral properties, the virus strain was multiplied in SF9 cell line grown in Grace's modified medium (Léry and Fédière, 1990). The AcMNPV was also multiplied on *Pectinophora gossypiella* (Vail *et al.*, 1972) and used as a source of infectious haemolymph.

Insect Rearing

Both *P. pectinophora* and *S. littoralis* were reared under laboratory conditions on semiartificial diets (Shorey and Hale, 1965; Couilloud and Giret, 1980). For *P. operculella*, small potato tubers were used as natural feeding source, while *S. cretica* was reared on a new semiartificial diet (unpublished data).

Larval Infection

AcMNPV produced on *P. gossypiella* larvae was purified and concentrated up to $60x10^5$ PIBs per milliliter. AcMNPV produced on the SF9 cell line was purified as follows: infected cells were scraped from the flask walls and centrifuged at 13000 g for 15 min. The pellets were resuspended in 1 ml tris (10 mM pH 7.8). The polyhedra were mixed with Triton-X at the final concentration of 0.1%, then used as infectious suspension on the artificial diet or potato tubers.

Newly hatched larvae of *S. littoralis* and *P. operculella* were exposed to contaminated diets while *2nd* and *3rd* instars of *S. cretica* and *P. gossypiella* were used for experimental infections.

Extraction and Analysis of Viral DNA

Infected larvae were homogenized in 50 mM Tris, 2 mM SDS pH 7.8, then filtered through cheesecloth. The suspension was purified by centrifugation on sucrose gradient 30-70% (W/W). Virions were liberated by alkaline treatment with Na_2CO_3 (60 mM final, pH 11). DNA was extracted by addition of 2% sodium lauroyl sarcosine and 200 µg/ml of proteinase K for 2 hrs at 50°C, followed by two extractions with phenol-chloroform-isoamyl alcohol (25/24/1), two phenol and two chloroform isoamyl (24/1) extractions. Finally, the DNA was precipitated with 2 volumes of glacial ethanol in presence of 1/10 volume of sodium acetate (3M) at -20°C overnight. Electrophoretic mobilities of different DNAs, digested with *Eco* R and *Hind* II restriction endonucleases and the migrations through 1% agarose gel were realized according to Maniatis *et al.* (1989).

Ultrastructural Studies

Larval tissues were prepared for electron microscope observations as follows: small pieces of fat body were fixed in 2.5% glutaraldehyde prepared in 0.01 M sodium cacodylate, then post-fixed in osmium tetroxide 1% in distilled water and embedded in Epon Araldite. Finally, ultrathin

sections were stained with uranylacetate saturated in 50% ethanol, then by lead citrate (Reynolds, 1963). Grids were examined through transmission electron microscope ZEISS EM10.

Preparation of Non Radioactive Nucleic Probe and Hybridization

Synthesis of non radioactive and dot blot hybridization for both AcMNPV and GVs were performed according to the supplier's protocol (Boehringer Mannheim).

RESULTS AND DISCUSSION

Four trials with AcMNPV ($10x10^3$ PIBs/ml) were carried out to infect *P. operculella*. No viral infection was observed. No late disease symptoms could be seen. On the other hand, infection of *P. operculella* with AcMNPV polyhedra, purified from infected cells of the SF9 cell line, caused 4% death and 13% abnormalities in larvae. Examination through optical microscope demonstrated the existence of inclusion bodies with cubic-shape and a significant number of granules (PTMGV) distributed in tested tissues were observed as well. Nucleic probes originated from both AcMNPV and PTMGV, separately applied, revealed that only 5% of larvae mixed infected with AcMNPV and PTMGV, while 75% of total insects were only infected with GV (results not shown). No AcMNPV infection alone was found. The presence of both GVs and AcMNPV could lead to the role, not yet precised, of PTMGV as an enhancing factor like other GVs previously studied (Yafeng *et al.*, 1989; Hashimoto *et al.*, 1991).

Concerning the trials for AcMNPV multiplication in PTM healthy larvae, only 3 larvae died at the 2*nd* instar 9 days post-infection showing high infection by AcMNPV (Fig. 1), while 40 larvae seemed to be normal and active at 4*th* to 5*th* instar and completed their life cycle. These low levels of AcMNPV multiplication in *P. operculella per os* was not correlated with the successful multiplication *in vitro* of the same virus on *P. operculella* cell lines (Léry *et al.*, 1995, in press). This contradiction could be due to one or more of the following reasons. Different levels of susceptibility to the viral infection within the insect populations could exist. The same probability could be expected in the viral population, especially because the detection of AcMNPV by the nucleic probe in the slightly abnormal alive larvae revealed its multiplication. Finally, this phenomenon could be simply due to the viral doses employed.

As previously reported by Scheepens and Wysoki (1989), no multiplication of AcMNPV was obtained in *S. littoralis* larvae.

Between 4 to 5 days post-infection of *S. cretica* by AcMNPV, the larval activity was remarkably reduced. Seven days post- infection, larvae seemed to be inactive and death began to occur. Examination of fat body tissue revealed the presence of polyhedra in the nuclei. Electrophoretic profiles of viral DNAs obtained from three infection replicates of *S. cretica*, analyzed with restriction endonucleases *Eco* RI and *Hind* II, compared to AcMNPV, indicated the identical DNA fragments sequences (Fig. 2). The examination of fat body tissues by electron microscopy revealed the presence of both viral particles of nuclear polyhedrosis type and *Sc*GV in the cytoplasm (Fig. 3).

The different results obtained on the 3 tested insects, regarding their susceptibility to AcMNPV could be considered according to two points of view. Firstly, if the tested host is not susceptible to AcMNPV (*S. littoralis*), it can play the important role of filter for the selection of genetically modified AcMNPV recombined with GV, in order to enlarge its host range. Secondly, if the insect is susceptible to AcMNPV (*S. cretica*), the tested insects will be added to the host list of AcMNPV, which could represent a possible alternative baculovirus for biological control after the achievement of necessary studies of the viral pathogenicity ad its persistence, particularly at the Delta and Nile valley.

In case of *P. operculella*, the fact that a little response to AcMNPV was observed, could permit us to consider the concept of filter.

In conclusion, using these different models, the potentialities of AcMNPV could be enhanced to play a new role in biological control programs, where the GV was the only possibility.

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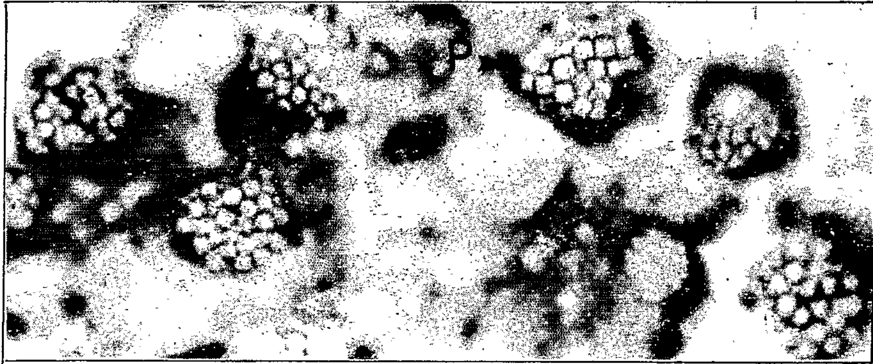

Fig. (1) Intranuclear polyhedral inclusion bodies (P) in *P. operculella* fat body cells.

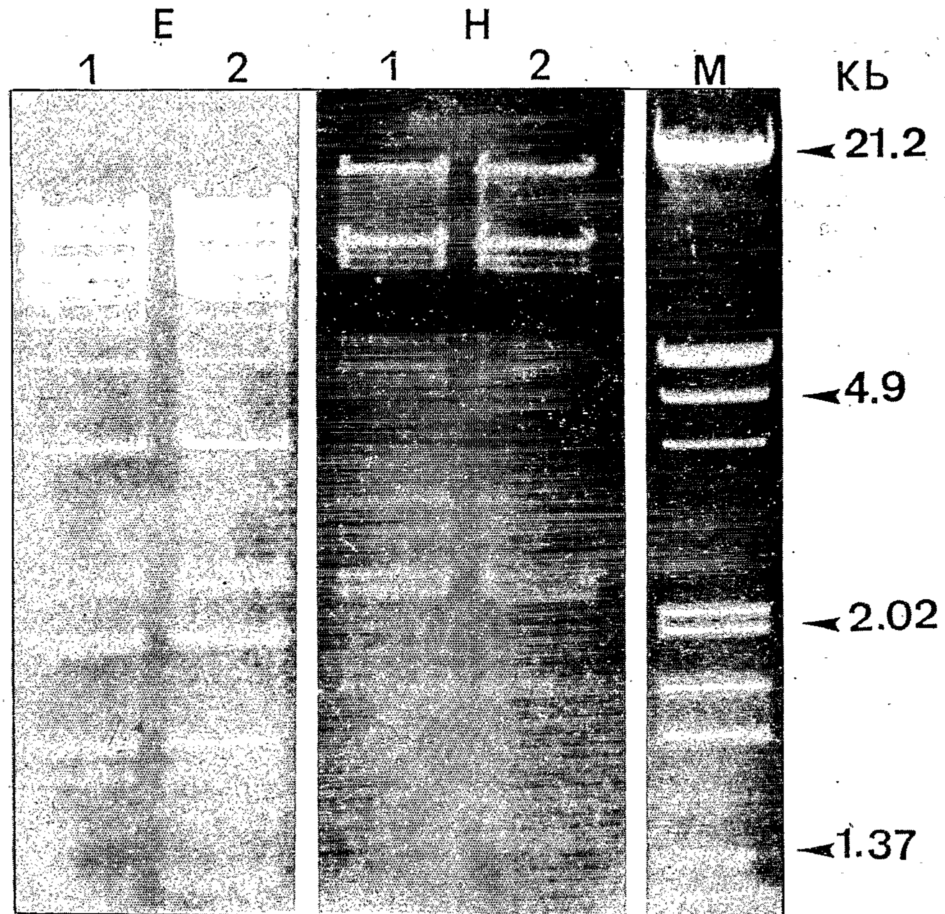

Fig.(2) Electrophoretic profiles of AcMNPV DNAs .
(1) AcMNPV multiplied in SF9.
(2) AcMNPV multiplied in *S. cretica.*
(E) AcMNPV digested with Eco RI endonuclease.
(H) AcMNPV digested with Hind III endonuclease.
(M) DNA marker III (Boehringer).

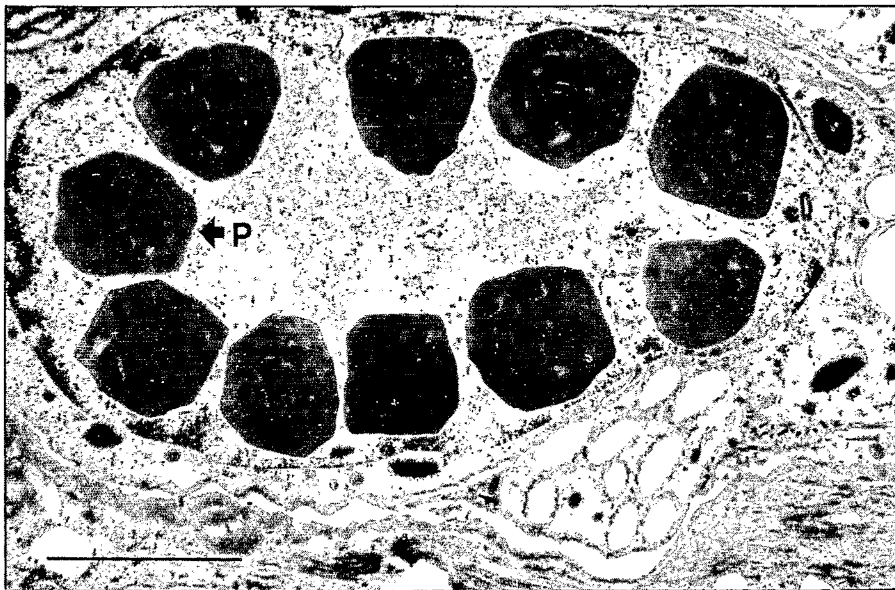

Fig. (3) Electron micrograph of *S. cretica* fat body infected with AcMNPV (bar represents 2μ).
(P) Polyhedral inclusion body.

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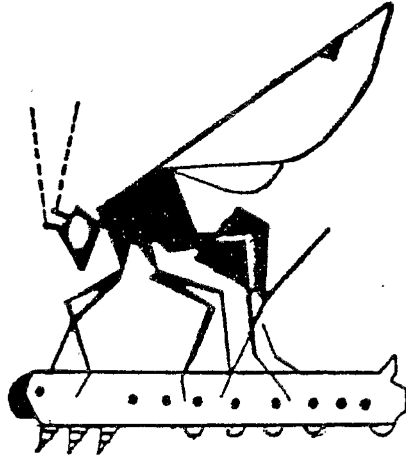

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Titre original : Host range of the *Autographa californica* multicapsid nuclear polyhedrosis virus (AcMNPV) in relation to granulosis viruses susceptible lepidopteran species in Egypt.

Titre en Français : Spectre d'hôte du virus de polyédrose nucléaire de *Autographa californica* (VPNAc), sur des lépidoptères susceptibles à des granuloses en Egypte.
(si le document est en langue étrangère)

Mots-clés matières : VPNAc, spectre d'hôte, granulose, Baculovirus, Spodoptera littoralis, Sesamia cretica, Phthorimaea operculella.
(10 au plus)

Résumé en Français : Le spectre d'hôte de la polyédrose nucléaire de *Autographa californica* a été étudié en Egypte sur 3 ravageurs susceptibles à des granuloses. Parmi les insectes testés *Spodoptera littoralis* ravageur du cotonnier, *Sesamia cretica* foreur du maïs, et *Phthorimaea operculella* la teigne de la pomme de terre, seuls *S. cretica* et *P. operculella* multiplient NPVAc. L'étude ultrastructurale et la détection du virus par sonde nucléique révèlent que *S. cretica* est très susceptible alors que 5% seulement d'infection a été obtenu avec *P. operculella*.
(150 mots maximum)

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