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A NEW RNA PICORNA - LIKE VIRUS IN THE COTTON PINK BOLLWORM *PECTINOPHORA GOSSYPIELLA (LEP.: GELECHIIDAE)* IN EGYPT

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A new virus infecting the pink bollworm *Pectinophora gossypiella* has been detected and purified from dead larvae collected from naturally infested cotton fields. The purified icosahedric virions measured 27 ± 1 nm in diameter and contained RNA genome. Three capsid proteins of 31.7, 32.6 and 47.4 Kd have been separated on polyacrylamide gel. The purified virus was not highly infectious to the host larvae revealed while the pupal period survived from infected larvae was significantly prolonged. The virus particles infecting the midgut cells are grouped in paracrystallin arrays. The virus was vertically transmitted through infected adults. The main characteristics of this virus place are quite relative to the Picornavirus group.

KEY-WORDS: *Pectinophora gossypiella,* RNA Virus, picorna-like virus, characterization, cotton, Egypt.

The cotton pink bollworm, *Pectinophora gossypiella* Saunders still considered as one of the most important worldwide spread pests of cotton. In Egypt, cotton fields represent about 1/6th of the total cultivated area, heavy losses are yearly recorded due to the attack of *P. gossypiella.* Chemical pesticides were applied for a long time without satisfactory results. So, alternatives to pesticides (e.g. selected varieties, use of pheromones and release of natural enemies, etc...) proposed to control the pest populations, are urgently required.

Some entomopathogens have been isolated from *P. gossypiella:* an infective bacteria, as well as two pathogenic protozoae which have been successfully transmitted and classified as *Mattesia grandis* Mc Laughlin and *Plistophora* sp. (Metalnikov & Metalnikov, 1932; Ignoffo & Garcia, 1965).

The first record of viral infection among *P. gossypiella* populations was reported by Smith & Rivers (1956). In 1966, Ignoffo & Adams mentioned the occurence of a reovirus (Cytoplasmic Polyhedrosis Virus) infecting the midgut epithelial cells. Further studies on the pathogenicity of this virus and its influence on adult longevity, fecundity and diapause were also reported Bullock *et al.* (1970), Bell & Kanavel (1976), Bell (1977). *Pectinophora gossypiella* is known as an alternative host to certain lepidopterous entomoviruses. The *Autographa californica* nuclear polyhedrosis virus (AcNPV) is considered to be highly infectious to pink bollworm larvae; field trials, using AcNPV formulations, had been carried out in Arizona to control this pest (Vail *et al.,* 1972, Bell & Kanavel, 1977).

Search for new viruses infecting *P. gossypiella* had been conducted in Egypt three years ago. In the present survey, the screening was principally carried out among dead larvae in the stored cotton bolls during their diapausing period. Through the permanent examination of dead larvae, a new free virus, proposed as PgV in the present paper, was isolated and purified; certain biological characteristics were also studied.

MATERIALS AND METHODS

LABORATORY REARING

A continuous rearing of *P. gossypiella* was carried out in laboratory using a semi synthetic diet (courtesy of Giret) modified from that of *Cryptophlebia leucotreta* (Couilloud & Giret, 1980). Such modification consists of the introduction of Pharmamedia (a cotton seed-dried protein nutrient) and the reduction of water content. The rearing was conducted in an incubator under constant parameters of temperature: 27 ± 1°C, relative humidity: 80% and photoperiod: 14 H.

VIRUS SCREENING

P. gossypiella larvae were directly collected from cotton bolls obtained from naturally infested cotton fields as well as from stored bolls on farms roofs which contains diapaused larvae. Dead larvae were individually examined for viral inclusion bodies detection. Smears have been prepared and stained with Methyl blue, then examined with light microscope through oil immersion lens. For screening of non-occluded viruses, the suspected larvae were collectively crushed in 0.01 M Tris SDS (pH 7.5), filtered through cheese-cloth. Suspension was clarified by 10 and 30 min. successive centrifugations at 3000 and 15000 rpm respectively using a Beckman J2 21 centrifuge JA 14 rotor. The supernatant was then sedimented at 35000 rpm for 2 hrs using a Beckman L7 ultracentrifuge Ti 55.2 rotor. The suspected virus particles in the resuspended pellet were purified on sucrose gradient (15-45% WW) using SW 28 rotor at 27000 rpm for 2 hrs. Suspected viral band was collected using a Beckman DU 70 spectrophotometer. Grids of concentrated virus suspension were negatively stained with PTA and then examined through a Phillips 400T electron microscope.

ANALYSIS OF VIRAL NUCLEIC ACID AND PROTEINS

Nucleic acid was extracted by phenol-chloroform after proteinase K action during two hours at 55°C and then precipitated in glacial absolute alcool. Analysis of PgV capsid proteins was conducted using SDS-PAGE according to Laemmli (1970). Number and molecular weights were estimated comparing their electrophoretic mobilities to those of low molecular weight electrophoresis calibration (Pharmacia Kit).

ULTRASTRUCTURE

Small fragments from infected insect tissues (midgut and fat body) were prefixed in 2% glutaraldehyde and post fixed in 1% osmium tetroxide, dehydrated and then embedded in pure epon. Ultrathin sections (500 Å) were stained and contrasted according to Reynolds (1963), then examined through transmission electron microscope.

SEROLOGY

Viral antiserum has been produced by three successive injections in rabbit, the first two injections were conducted using 0.5 ml of 2.0 OD purified virus mixed with 0.5 ml

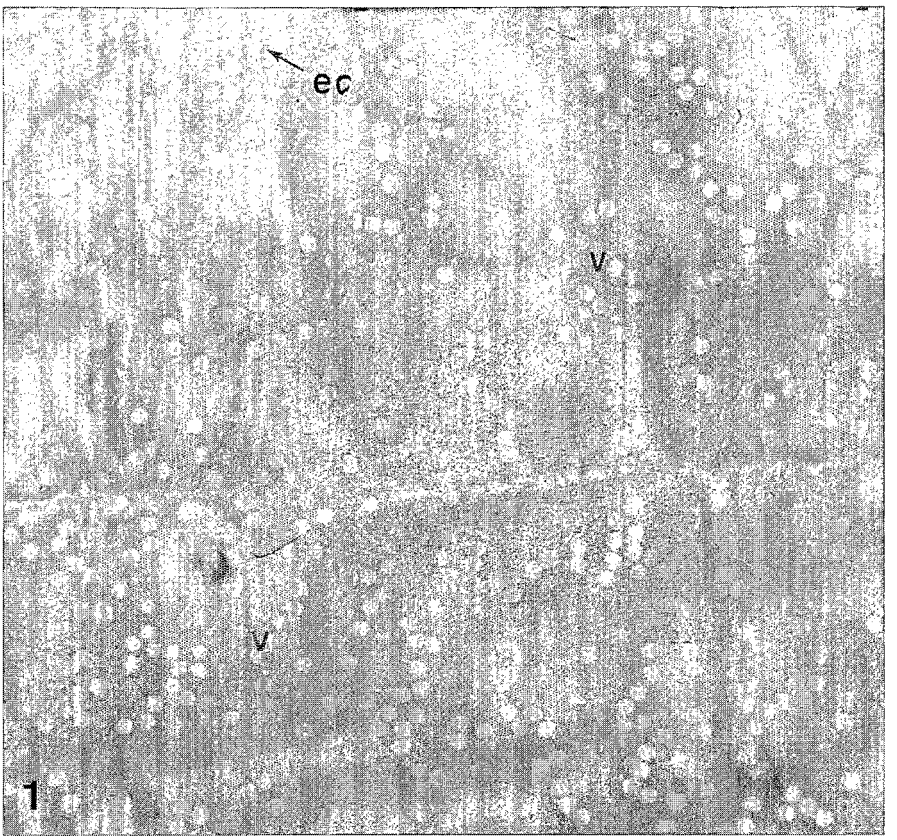

Fig. 1. Purified virions of *P. gossypiella* virus. v: virus particles, ec.: empty capsid. E.M. 74000 X.

Freund's adjuvant, and the third one with 1 ml of 1.0 OD four weeks later. The antigen-immunserum reaction was detected using the double diffusion technique in 1% agarose gel described by Outcherlony (1958). The indirect ELISA test was also applied for the detection of virus in infected tissues according to Voller *et al.* (1976).

RESULTS

The absorption 260/280 ratio of virus particles is 1.74 indicating a nucleic acid content of isolated particles.

As shown in fig. 1, the negatively stained highly purified virus particles were icosahedral in shape and measure 27 ± 1 nm in diameter. Few particles were interiorly stained by the PTA showing the viral capsid. Virions were identical in shape and size.

VIRAL PROTEIN AND NUCLEIC ACID

Electrophoresis of viral proteins revealed three major proteins of 31.7, 32.6 and 47.4 Kd (fig. 2) while no minor protein were observed. Extracted nucleic acid, digested by both

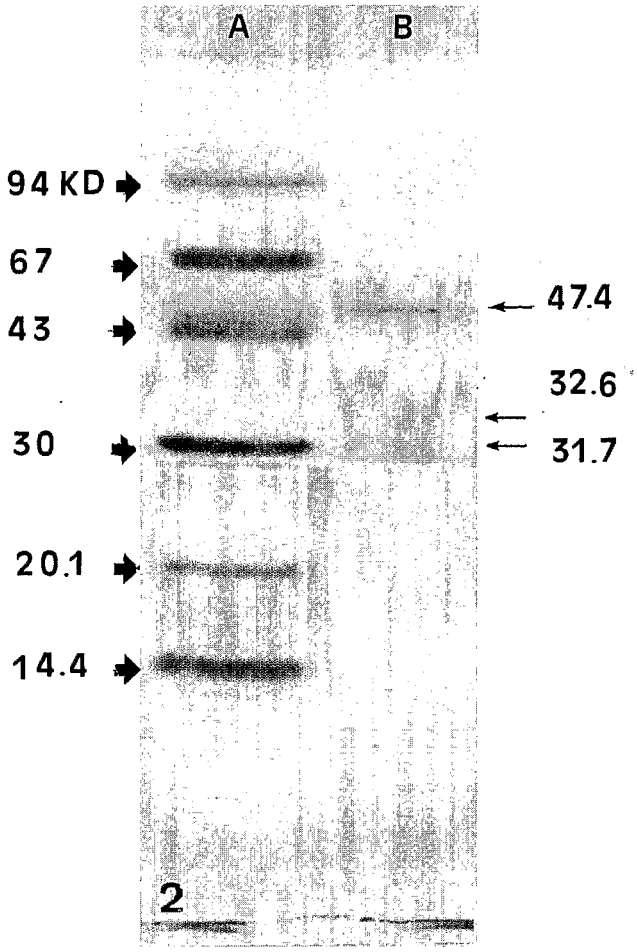

Fig. 2. Viral protein of *P. gossypiella* through SDS-PAGE. Lane A: low molecular weight marker proteins. Lane 2: Viral capsid proteins.

DNAse and RNAse then electrophorised under denaturing condition, revealed the resistance to DNAse which proves the RNA single stranded nature of the viral nucleic acid.

ULTRASTRUCTURE

The PgV particles were principally detected in the cytoplasm of the midgut epithelial cells. As shown in fig. 3, virus particles were rarely and irregularly scattered in the cell, the effective presence of virus particles was observed under the form of aggregates with variable volumes inside the vesicles. The intact single or double membraned vesicles have spherical or ovoid shapes, the viroplasmic regions were also present inside the vesicles and the virus particles were either densily scattered or found in paracrystalline arrays. The infected tissues were poor in cellular cytoplasmic substances in spite of the existence of

giant mitochondria and rough endoplasmic reticulum (fig. 5). The cytoplasm seems to be disrupted, it is probably due to the conditions of tissue fixation. Fibrilar structures (fig. 4), corresponding to the vesicles in volume, were frequently observed in the cytoplasm of adult infected tissues. The fibrilar structures, which derived of outline membranes, were not homogenous in fibrilar substance, the regions which were less dense or without fibers were occupied by aggregates of virus particles. It is probably the origin of the vesicle formations regarding the expansion of virus aggregates which replace gradually the fiber contents (fig. 4).

IMMUNOLOGY AND VIRAL INFECTIVITY

The virus was serologically detected using the rabbit antiserum. Through double diffusion gel, the positive reaction was detected between PgV and its antiserum while no immunological reactions were observed with the Picornaviruses of *Sesamia cretica* (Fedière *et al.,* 1991) and *Latoia viridissima* (Fédière *et al.,* 1990). The diluted antiserum reacted with PgV suspension up to 1/64, this indicates that the antiserum was capable to be used for ELISA test in order to detect the virus among individually infected larvae.

The virus did not appear to be highly virulent to *P. gossypiella* larvae. In bioassay tests, virus suspension was applied to 2nd and 3rd larval instars as 20 μl of viral suspension *per* larva. The applied concentrations of purified virus were estimated as 1.7 OD for L3, 1.0 OD, 0.1 OD and 0.01 OD for L2. This respectively corresponding about 3.4×10^8 virions/larva for the first test and 2.0×10^8, 2.0×10^7, 2.0×10^6 virions/larva for the second one.

Only the concentrated suspension was quite virulent to 2nd larval instar; the concentration of 1.0 OD induced 20% mortality during 18 days post-infection, while no mortality was obtained with other applied suspensions. For the 3rd instar, the effective concentration was only that of 1.7 OD which induced 36% mortality during the same delay.

Among the survived individuals, 25% of treated larvae continued their development to pupal stage which survived 40 days compared with 20 days for 95% of control pupae.

DISCUSSION

A quite number of non envelopped small RNA viruses was characterized and classified among Picornaviridae, Nodaviridae, and Tetraviridae (Reavy & Moore, 1982). In spite of the extensive research for *P. gossypiella* entomoviruses, the Pg non occluded RNA virus is considered as the first record of this group of viruses infecting the pink bollworm. The comparison of PgV major proteins and those of Picornavirus reference type (24 to 41 Kd) shows certain correspondence (Matthews, 1982). Many insect picorna-like viruses are known to have one heavier protein than the reference recorded in Matthews (Moore & Eley, 1991). Some authors reported in this case the existence of VPO protein which is represented in gel as VP4 and VP2 by cleavage. The PgV virion localized in paracrystalline arrays inside the cytoplasmic vesicles were observed among other Picornaviruses especially that of the *Gonometa podocarpi* (Longworth *et al.,* 1973, Henry & Oma, 1973, Moore, 1985, and Chao *et al.,* 1985). In spite of the lack of cytoplasmic substances, the existence of giant mitochondria and rough endoplasmic reticulum reflects the activity of infected cells. The later observation was reported by Laubscher *et al.* (1992) in the aphid *Rhopalosiphum padi* infected by the Lethal Paralysis Virus. Slight information concerning the lethal effect of insect picornaviruses is known. However, the research for isolating new entomoviruses was mainly directed to their pathogenic effect, in the field of insect pathology, it is

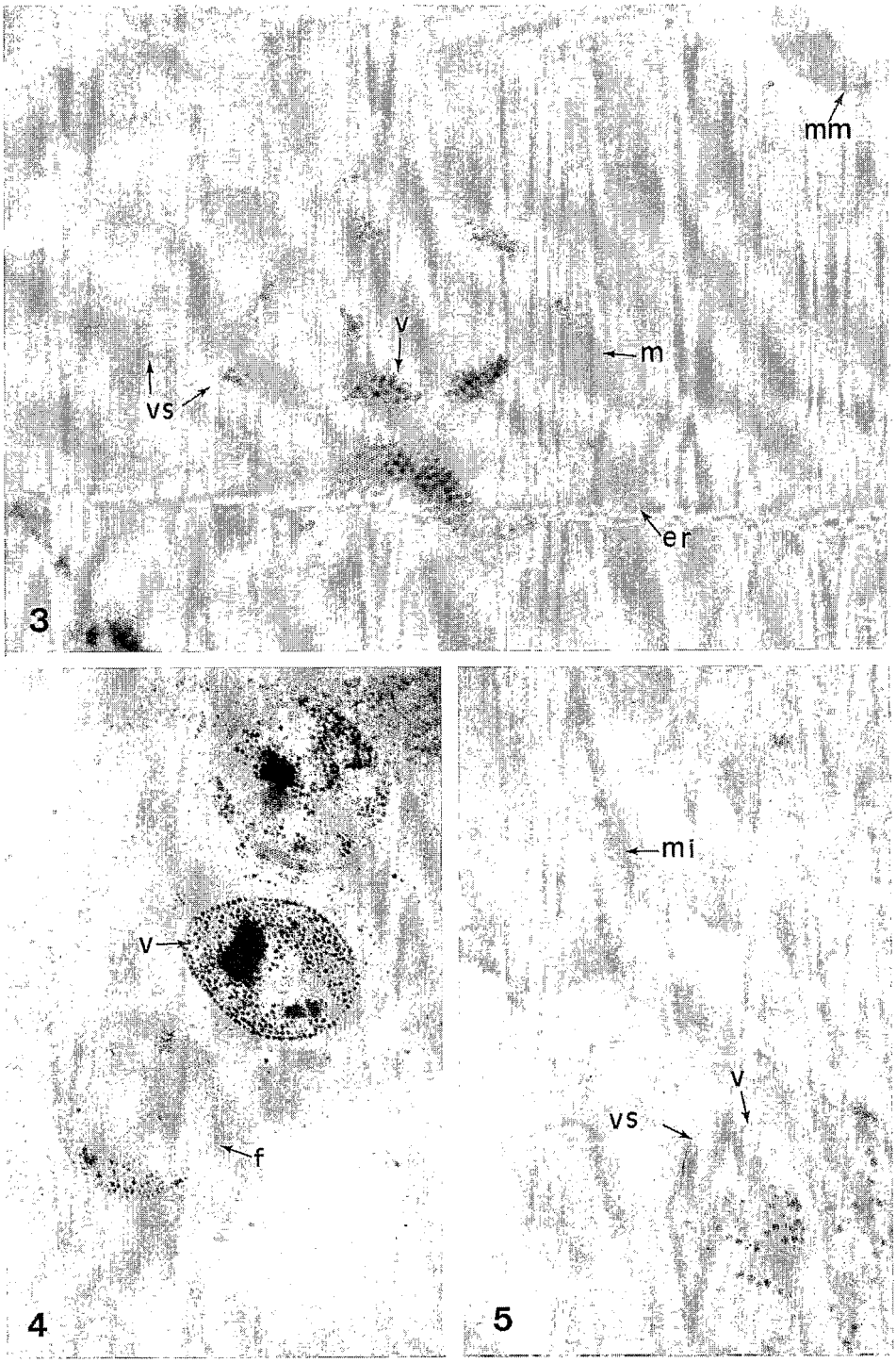

Fig. 3. Ultrathin section showing viral particles in the cytoplasm of the larval midgut epithelial cells. v: virion, vs: vesicle, m: vesicular membrane, mm: double vesicular membrane, er: endoplasmic reticulum, 50000X.

Fig. 4. Electron micrograph indicating the formation of viral particles in the fibrilar structure of adult midgut epithelial cells. f: fibrilar structure, 32000X.

Fig. 5. Development of virus particles inside the vesicle of larval midgut cells. v: virion, vs: vesicle, mi: mitochondria, E.M. 50000X.

well known that some viruses exist either in the environment or in laboratory rearing which are not pathogenic to their respective hosts. In this case, the host insects reach their complete biological cycle without any sign of pathological symptoms (L'heritier, 1954; Stolz & Vinson, 1977; Mialhe, 1982). Recently, Moore & Eley (1991), reviewed the small RNA insects viruses which resulted or not in the pathological symptoms. Chao *et al.* (1985) and Muscio *et al.* (1988) described the existence of picorna-like viruses in apparently healthy individuals respectively of *Pseudoplusia includens* and *Triatoma infestans.* These infections were considered as associated or non pathogenic viruses.

The experimental infection by *P. gossypiella* virus, which induce a moderate larval mortality, indicate the non acute pathogenic effect of this virus. Through laboratory observations, the virus is always existing within adult populations which continue their life cycle either in laboratory rearing or among field populations (unpublished data).

Finally, this form of associated viruses could play an important role as a regulating factor of pest populations, so further studies and observations are required to determine the role of PgV and the mechanism of its transmission through successive generations.

RÉSUMÉ

Un nouveau « Picorna-like » virus à ARN isolé chez la teigne du cotonnier *Pectinophora gossypiella* (Lep. : Gelechiidae) en Egypte

Un nouveau virus infectant le ver rose du cotonnier, *Pectinophora gossypiella,* a été isolé et purifié à partir de larves mortes récoltées en champ de coton. Les virions purifiés de 27 ± 1 nm de diamètre, ont une forme icosaédrique et contiennent un génome à ARN. Trois protéines capsidaires de 31,7, 32,6 et 47,4 Kd ont été trouvées dans les virions. Le virus purifié n'est pas hautement infectieux pour les larves, cependant la durée du stade chrysalide est supérieure chez les lots infectés artificiellement. Dans les tissus atteints, les virions sont dispersés dans le cytoplasme des cellules de l'intestin ou bien groupés en amas paracrystallins de même type que chez d'autres insectes infectés avec des picornavirus. Les adultes malades transmettent ce virus à leur descendance. Par sa forme, sa taille et ses caractéristiques chimiques, ce virus libre peut être placé près des Picornavirus d'insectes.

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