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

A. MONSARRAT(1), S. ABOL-ELA (1), I. ABDEL-HAMID (1), G. FEDIERE (1), G. KUHL(2), M. EL HUSSEINI (3) & J. GIANNOTTI (1)

(1) French/Egyptian Virology Lab., Fac. of Agriculture, Cairo University, Giza, Egypt.
(2) Station de Recherches INRA-CNRS 30380, St Christol-les-Alès, France
(3) Biological Control Lab., Fac. of Agriculture, Cairo University, Giza, Egypt

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, charac-
terization, 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 occurrence 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