A Granulosis Virus of the Peach Fruit Moth, *Carposina niponensis* WALSINGHAM (Lepidoptera: Carposinidae)

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Description of a newly discovered granulosis virus of the peach fruit moth *Carposina niponensis* and histopathological observation of the infected larva were reported in this paper. The oval capsules were approximately 480 nm long and 300 nm wide, while the virus rods were 265 nm long and 40 nm wide. Light microscopic observations of the infected larvae suggested that the fat body, tracheal matrix, Malpighian tubule, epidermis and midgut were the tissues susceptible to the virus. A cross-transmission test of the virus to larvae from four Tortricidae species, *Adoxophyes* sp., *A. arana fasciata*, *Archips breviplicans*, and *Homona magnanima*, was unsuccessful.

INTRODUCTION

The larva of the peach fruit moth, *Carposina niponensis* WALSINGHAM is one of the pests most destructive of deciduous fruits such as apples, pears and peaches in Japan. Control of this fruit borer depends mainly on the application of chemical insecticides. A fungus, *Pasciomyces fumosoroseus*, has been known as the sole microbial control agent for this pest (AKASHI and SEKIGUCHI, 1952; SEKIGUCHI, 1959).

In November, 1971, nine diseased larvae of the peach fruit moth were collected from injured apples obtained from trees in the fields of the Akita Fruit Tree Research Station, HIRAGA, Akita Prefecture. The diseased larvae had an intensely yellow body color compared with normal larvae, their body segments were fatted and the hemolymph became turbid. Under an electron microscope, many oval bodies characteristic of granulosis were observed in the larval fluid. Among the so-called fruit borers, the codling moth, *Laspeyresia pomonella*, and the oriental fruit moth, *Grapholitha molesta*, are susceptible to a granulosis virus of *L. pomonella* (TANADA, 1964; IGNOFFO and HINK, 1971). However, we are aware of no record of virus diseases of the peach fruit moth. This paper describes some features of a newly discovered granulosis virus of the peach fruit moth and the histopathological observations of the infected larvae.

MATERIALS AND METHODS

The adults of the peach fruit moth which emerged from the injured apples obtained from the orchards of the Akita Fruit Tree Research Station were allowed to oviposit on the surface of apples hung in a net room. These apples were placed in glass vessels

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(15 cm in diam., 9 cm in height), and kept at regulated temperature (25 °C) and illumination (for 16 hr per day). The larvae hatching fed on the same apples. The smaller tea tortrix Adoxophyes sp. (species name pending), the summer fruit tortrix Adoxophyes orana fasciata, the Asiatic leaf roller Archippus breviplicatus and the tea tortrix Homona magnanima were reared on a slightly modified artificial diet for Adoxophyes sp. (TAMAKI, 1966).

The virus was obtained from the above-mentioned diseased larvae of C. nipponensis. The virus inoculum for the cross-transmission test was prepared by mercerating one infected matured larva in 100 ml of distilled water. For inoculation of the peach fruit moth, the moths were allowed to oviposit in a net room on young apples which had been dipped in a virus suspension containing a small quantity of Triton X-100 (1:5,000) as a wetting agent and then dried. The larvae hatching from these eggs bit the surface of the apple contaminated with the virus and bored into the fruit. The virus inoculation of the larvae of four Tortricidae species was performed by the following method. The apple leaves which had been smeared with the virus suspension and air-dried were placed together with sterilized egg masses of each species in glass vessels (9 cm in diam., 5.5 cm in height) and held at 25 °C for 7 days. After hatching larvae fed on the virus-contaminated leaves, they were removed individually to vials (1.2 cm in diam., 6 cm in length) containing an artificial diet.

For electron microscopy, the fat body tissues of infected larvae were fixed in 3% glutaraldehyde in phosphate buffer (pH 7.2) for 1 hr and post-fixed in 1% osmium tetroxide in s-collidine buffer (pH 7.3) for 2 hr. The dehydrated tissues were embedded in Epon resin and cut with a Porter-Blum ultramicrotome. The sections were double-stained with uranyl acetate and lead citrate. Observations were made with a Hitachi HU-11D electron microscope. For light microscopy, the diseased larval bodies fixed in Bouin's fluid were embedded in paraffin. The sections cut at about 7 microns were stained by Feulgen's technique with fast green FCF as a counterstain and examined under a phase-contrast microscope.

For measurement of the sizes of capsules and virus particles, the diseased larvae triturated were filtered through four layers of cheesecloth. The suspension was partially purified with differential centrifugation (at 3,000 rpm for 10 min and 12,000 rpm for 30 min). To liberate the virus particles, the purified capsules were treated with a weak alkaline solution (a mixture of 0.1 M Na₂CO₃ and 0.1 M NaCl) for 3 hr at room temperature. The solution was centrifuged at 5,000 rpm for 10 min and at 14,000 rpm for 40 min. Each pellet was resuspended in a small volume of distilled water. The capsule specimens were shadowed with platinum-palladium alloy and the virus specimens were negatively stained with 2% phosphotungstic acid in phosphate buffer (pH 7.0).

RESULTS AND DISCUSSION

Examined with an electron microscope, almost all capsules were oval in shape but occasionally abnormally shaped capsules were found (Figs. 1, 12, 13). The average size and standard error of the normal capsules were 480±15 nm in length and 300±20 nm in width. The virions were rod-shaped, 265±5 nm long and 40±15 nm wide.

Under the light microscope, the cells of the fat body and epidermis were very hypertrophied and the Feulgen positive material was observed mainly in the nuclei of the cells of the fat body, epidermis, midgut, tracheal matrix and Malpighian tubule (Figs.
Granulosis Virus of the Peach Fruit Moth

Fig. 1. Electronmicrograph of the capsules of the granulosis virus of the peach fruit moth (× 10,000 arrows indicate polystyrene latex particles with diameter of 109 nm)

Fig. 2. Electronmicrograph of the virions liberated from capsules by alkaline treatment (× 16,000, arrows indicate the PSL particles)

Figs. 3–9. Light micrographs of the various tissues of the larva of the peach fruit moth stained by Feulgen’s technique with fast green FCF (× 540). Figs. 3 and 4: Fat bodies infected with the granulosis and normal; Figs. 5 and 6: Infected and normal epidermal cells, Figs. 7, 8 and 9: Infected Malpighian tubules, tracheal matrix and midgut.

3–9). These nuclei were much larger than those of the healthy ones. These histopathological changes are similar to the pattern of some Lepidoptera insects infected with granuloses, which has been reported by Huger and Krieg (1961), Tanada and Leutenegger (1968), Watanabe and Kobayashi (1970) and Oho et al. (1974). Therefore, it is suggested that these five tissues were susceptible to this virus infection.

The electron-microscopic observation of the fat bodies revealed that some of the capsules in the cytoplasm were surrounded by several with membrane-like material (Fig. 10). This material showing the myelin figure might be a kind of cytolysosome (Fig. 10, arrow). Similar lysosomes enclosing several virus particles were observed by Kobayashi (1971) in the gut cells of silkworm larva in the progress of the infection of a cytoplasmic-polycytophrosis virus. The cytolysosome in the infected cells of the larva of the peach fruit moth, enclosing not only the virions but also the inclusion bodies, is
Figs. 10–13. Electronmicrographs of the fat-bodies of the larvae of the peach fruit moth infected with the granulosis virus (Figs. 10–12: $\times 20,000$; Fig. 13: $\times 10,000$). C: Capsule; CL: Cytolysosome shown by arrows. The membrane of the cytolysosome disappeared in the process of the maturation of the capsules from Figs. 10 to 13.

probably due to the small size of the capsules compared with the polyhedra. The cytolysosome in the early stage of infection would be the result of a resistant reaction in the host cell against the heterogeneous substance. In the final stage of infection, the cytolysosome was crumbled away and the capsules were uniformly distributed all over the cells (Figs. 12, 13).

The cross-transmission tests of the virus to larvae of four Tortricidae species, Adoxophyes sp., A. orana fasciata, Arachips breviplicatus and Homona magnanima were not successful, but the peach fruit-moth larva was susceptible. This result suggests that the virus is not derived from these Tortricidae pests of the apple orchard.

Although the similarity of features between the granulosis viruses of the codling moth and the peach fruit moth must be investigated further, the utility of this newly discovered granulosis is anticipated to be a promising microbial control agent. For the codling moth, which closely resembles the peach fruit moth in its damaging behaviour, it has been proved that application of virus suspension to apple trees reduces the population and prevents excessive damage to the apples (FALCON et al. 1968). For further investigation of the applicability of this virus to microbial control, it is essential that a mass rearing method for this insect be established.

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REFERENCES


