Biological Control

Isolation and Identification of a New Virus Infecting *Pandemis pyrusana* in Apples

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A laboratory culture of the virus killing *Pandemis pyrusana* (PLR) at the TFREC was successfully established and used for further studies. Preliminary evaluation of symptomology and morphological examination of Transmission Electron-Microscopy of thin sections of virus capsules in PLR tissues confirm that this virus is a granulosis virus. Surveys in orchards at the TFREC have indicated that this virus caused from 10.4-72.2% mortality of field collected, non-parasitized PLR during the last 4 generations (spring and summer 1997 and 1998). Purification techniques have been devised and a large amount of purified material is being stockpiled for further testing. Preliminary evaluations have strongly indicated that this virus has no effect on obliquebanded leafroller (OBLR) or *Xenotemna pallorana* and is specific to PLR. These results suggest that this virus holds a great deal of promise as specific control tactic for PLR.

Results

Field Infection of *P. pyrusana*

Populations of PLR were surveyed in 1997 and 1998 at the TFREC to determine levels of infection and associated mortality in field leafroller populations. Larvae were collected from unsprayed blocks at the TFREC during the spring and summer generations of PLR. These larvae were returned to the laboratory and placed on artificial diet to allow them to develop to adulthood or die of viral infection. Mortality due to viral infection is easy to identify by the larvae turning an opaque milky white/yellow and becoming bloated before death. Mortality of non-parasitized PLR due to infection was 10.4 and 62.5% for the spring and summer of 1997 and 72.2 and 25% for the spring and summer generations in 1998, respectively.

Initiation of Virus Culture

Infected PLR were isolated from field collections in August 1997. Infected PLR were placed in 15 ml of distilled water and macerated with metal forceps. The resulting suspension was evenly distributed on the inside surface of ca. 25 diet cups (90 ml) in which a 1 cm thick layer of leafroller diet had been placed. Six 4th-5th instars were placed in each cup and allowed to feed on the virus-coated diet. PLR were then reared at room temperature and checked for expression of viral infection. Initial observations of symptoms of infection occur about 6 days after exposure, and mortality occurs from 10-15 days after exposure. Culturing of the virus has continued using this technique.

TEM Visualization of Purified Viral Capsules

Purified viral capsules were examined using TEM and a negative staining procedure. Purified viral capsules were mixed 1:1 with 1% phosphotungstic acid, pH 8.5. A small droplet of

this mixture was placed on a carbon-coated grid and excess solution drawn off with a piece of filter paper, leaving a thin film behind. The film was allowed to dry and then viewed with a JEOL transmission electron microscope. Visualization of purified virus capsules showed typical morphology associated with granulosis viruses. The capsules viewed were of characteristic ovocylindrical shape produced by the protein occluding the viral particle. Average size of capsules seen were approximately 327 nm ±31.5 nm long by 185 nm ±28.2 nm wide (n=50), well within the reported range of granuloses (300-500 nm in length by 120-300 nm in width) though on the small side. This initial morphological examination therefore indicated that the virus infecting *P. pyrusana* was a granulosis virus as speculated.

Thin-Sectioning of Virus Capsules

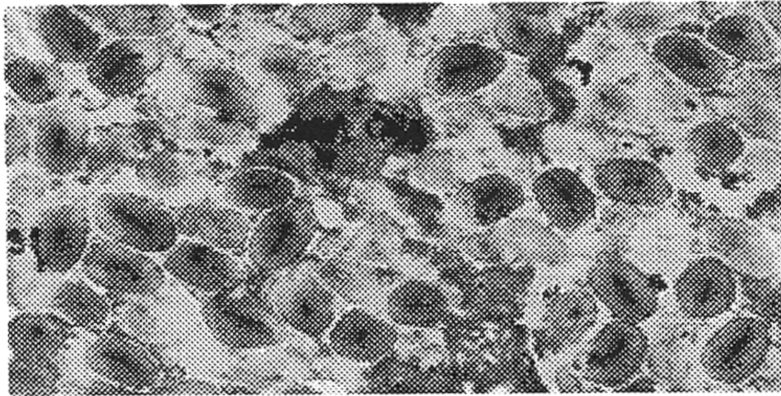
Infected insects 9 days post-infection were examined in this portion of the project. Insects were dissected in a bath of 2% glutaraldehyde/0.1M Cacodylate buffer and midguts were removed. Midguts were then fixed in 2% glutaraldehyde/0.1M Cacodylate buffer overnight at 4°C. Samples were post-fixed in 2% osmium tetroxide, run through an alcohol dehydration series and embedded in Spurr's resin. Thin sections were obtained using a diamond knife and sections were viewed on a TEM. Thin sections revealed extensive amounts of virus within *P. pyrusana* tissues. Longitudinal and transverse sections of viral capsules were also observed and were characteristic of previously recorded images from other granuloses. The crystalline lattice of the occlusion protein of the capsule, typically seen in granuloses, was observed in thin sections at high magnification. Also readily observable was the nucleoprotein of the virus itself, seen as a slightly curved rod in the center of the capsule in longitudinal sections. Around the viral rod could be seen, in both longitudinal and transverse sections, the viral envelope associated with the nucleocapsid of the virus. TEM confirms morphologically that the virus infecting *P. pyrusana* is a granulosis virus (PpGV).

Preliminary Screening of PpGV Infectivity Against PLR, OBLR and *Xenotemna pallorana*

Five infected PLR were macerated in 20 ml water and 0.5 ml of the virus suspension placed in each of 10 diet cups containing a l cm layer of artificial leafroller diet. To each treated cup 5 PLR were added. As a control, 10 cups had only water added before addition of larvae. Larvae were evaluated after 3 weeks to determine how many larvae had survived to pupate vs. those which had died or were near death. This process was replicated three times for PLR, OBLR and *X. pallorana* for a total of 150 larvae of each species exposed to each treatment.

This dose of PpGV caused 100% mortality of the PLR larvae and no detectable mortality to either OBLR or *X. pallorana.* The few OBLR and *X. pallorana* individuals which died did not exhibit any of the symptoms associated with PpGV infection.

	% larval mortality	
Leafroller species	Virus	Control
P. pyrusana	100	0
C. rosaceana	2.0	3.3
X. pallorana	1.3	0



Pandemis pyrusana **Granulosis Virus in infected tissues as viewed by Transmission Electron Microscopy**