

ACTIVATION OF DIFFERENT LATENT VIRUSES ON *PHTHORIMAEA OPERCULELLA* (ZELLER) (LEPIDOPTERA: GELECHIIDAE) USING THREE TYPES OF STRESSORS

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Abstract – The detection of different viruses in the potato tuber moth (PTM) *Phthorimaea operculella* *in vivo* and *in vitro* magnified the importance of understanding their nature and role. To characterize these viruses, it was necessary to increase their replication. It was possible to induce them using three types of stressors: (i) experimental infection with heterologous viruses, (ii) overcrowding and (iii) intoxication with a chemical substance. Both larvae of PTM collected in the field and reared in the laboratory were susceptible to these three kinds of stressors. It confirms the hypothesis of the existence of latent viruses in *Ph. operculella* which are related to the families Picornaviridae, Nodaviridae and probably Parvoviridae.

Key words – Potato tuber moth, *Phthorimaea operculella*, latent virus activation, Densovirus, Picornavirus, Nodavirus.

INTRODUCTION

There are many recent reports concerning the detection of adventitious virus infections in insects. These endogenous viruses have been considered occult or latent, and their pathogenicity and/or the interaction with other viruses has not been established (GRANADOS *et al.* 1978). The reason why some viruses remain inapparent and persistent in some hosts and cause lethal effects in other species is not understood (MOORE & TINSLEY 1982). While it has been suggested that such infections can be activated by stress factors such as low temperature, overcrowding and unsuitable diet (SMITH 1963; LONGWORTH & CUNNINGHAM 1968), it seems difficult to test that experimentally in every case (McKINLEY *et al.* 1981).

In our laboratory, two viruses had been isolated from established cell lines of the potato tuber moth (PTM) *Phthorimaea operculella* (Zeller) deriving from embryos (LÉRY *et al.* 1998). The first one was related to the Nodaviruses, with 30 nm in diameter, an icosahedral capsid and a bisegmented RNA genome, and the second one related to the family Parvoviridae resembling densovirus group, with 25 nm in diameter, an icosahedral capsid and a DNA genome. Since they were detected *in vitro*, it was necessary to study their presence *in vivo*, in their natural and expected source. Since they had a very low replication

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rate it was also necessary to activate them by some kind of stressors in order to obtain sufficient amounts of virus for their subsequent analysis and study. In the present study, we will describe the activation of three types of latent viruses in *Ph. operculella*, related to families Picornaviridae, Nodaviridae and probably Parvoviridae.

MATERIALS AND METHODS, RESULTS AND DISCUSSION

Characterisation of latent viruses activated by another virus

In the way to study the latent viruses in *Ph. operculella*, the larvae were susceptible to be induced by the *Junonia coenia* densovirus (JcDENV) gift from Dr J.-C. Veyrunes (St Christolés-Alès, France) as a heterologous virus infection at concentrations of 0.6, 1, 2, 4, 6, 8 and 10 optical densities (ODs), mixed with 0.1 % Triton X-100. We assayed the third generation of the PTM in the laboratory. These concentrations were sprayed on the potatoes which were exposed later to the newly hatched larvae of PTM. The mortality responses gradually increased according to the virus concentration as shown in Fig. 1A.

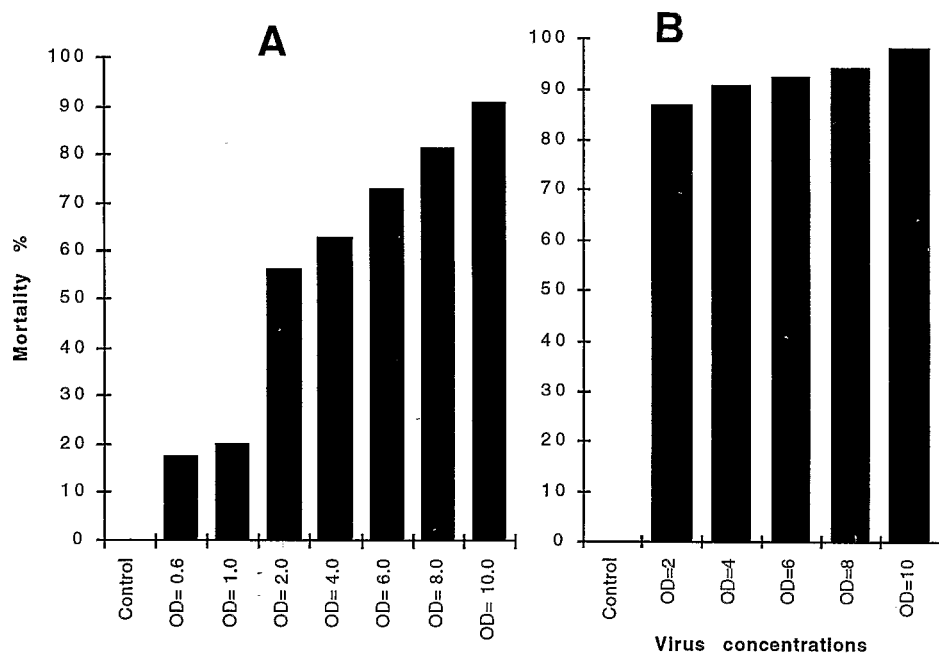


Figure 1. – Effect of different concentrations of JcDENV on the third (A) and the tenth (B) generation of *Phthorimaea operculella* larvae reared in laboratory. OD = optical density of the virus concentration.

Dead larvae were collected daily after the fourth post-treatment day, as the larval development proceeded (from L₁ to L₄) the larvae became less affected by the treatment. These dead larvae were refrigerated and then homogenized in 0.05 M Tris, pH 7.8 and the suspension was clarified by filtration through thin layer of cotton, then centrifuged at 1500 × G for 10 minutes. The resulting supernatant was centrifuged at 100 000 × G for 2 hours. The pellet was resuspended in Tris and then layered on 15/45 (w/w) sucrose gradient

Virus activation on Phthorimaea operculella

and centrifuged at $120\,000 \times G$ for 2 h 30. Virus bands were recovered and pelleted as above. The pellet was resuspended in Tris and examined by electron microscopy (EM) after negative staining in 2 % uranyl acetate.

The presence of two particle sizes were revealed. The first one, 30 nm in diameter was related to the family Picornaviridae and the second one, 25 nm in diameter, was related to the Densoviruses of Parvoviridae. The protein analysis using 10 and 12 % SDS-PAGE, according to LAEMMLI (1970), revealed the presence of six polypeptides. Four of them: 90, 63, 53 and 43.5 kDa were related to the JcDNV (but in few amounts), and the two others: 35 and 33 kDa were related to the picornavirus, as a dominant one (Fig. 2A).

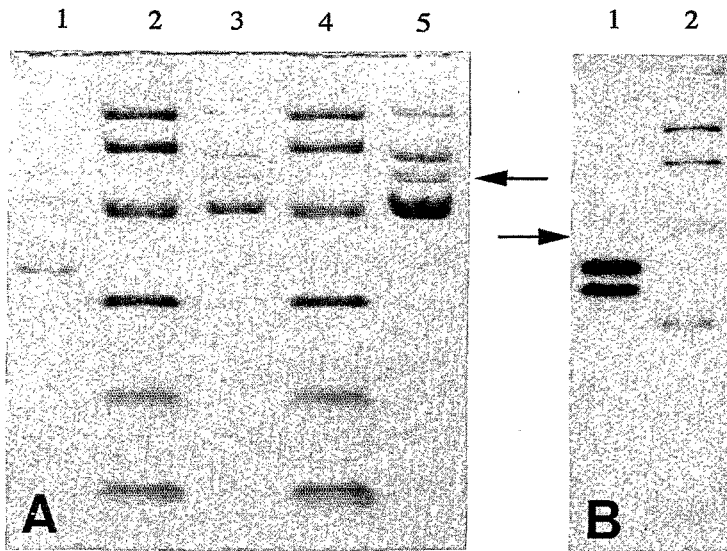


Figure 2. — Electrophoregramme of viruses purified from *Phthorimaea operculella* larvae in 12 % polyacrylamide gels. **2A:** Activation by JcDNV: 1- Mixed viruses obtained directly from *Ph. operculella*, 2 & 4- Markers, 3- JcDNV, 5- Mixed DNVs obtained after amplification on *Spodoptera littoralis* larvae, arrow indicate the additional polypeptide corresponding to the PoDNV. **2B:** Activation by Crowding: 1- Mixed Picorna-like virus and Nodavirus, 2- Marker, arrow indicates the unique polypeptide of Nodavirus.

This viral mixture was used to infect *Spodoptera littoralis* (Boisduval) larvae as a susceptible host for JcDNV, in order to select the densovirus for a subsequent analysis. The viruses recovered from dead *S. littoralis* larvae, were purified as described before. The EM examination revealed the presence of a denso-like virus alone. The protein analysis gave five polypeptides. Four of them: 90, 63, 53 and 43.5 kDa were related to JcDNV and the additional band, near 64 kDa, revealed the presence of another virus. The *S. littoralis* larvae infected by the JcDNV alone as a control, resulted in the replication of JcDNV only and four polypeptides could be found.

The DNA of this mixture, named PoDNVs, was extracted by a phenol/chloroform/isoamyl alcohol technique (SUMMERS & SMITH 1987) and digested by three restriction

endonucleases: BamHI, HaeII and BglII (Table I) in a comparison with JcDNV which has a genome of 5.85 kilobase pairs (kb). BamHI recognized the same restriction sites for both viruses and gave three bands of 5.25, 0.31 and 0.29 kb. HaeII could cut the JcDNV in two fragments of 5.35 and 0.5 kb, but the PoDNVs in five fragments, two of them related to JcDNV, and three additional fragments of 3.3, 2.05 and 0.5 kb, related to another densovirus. This was confirmed using the BglII enzyme which could not recognize JcDNV, but could also digest PoDNVs, giving one fragment of 5.85, corresponding to the JcDNV, and two more bands of 4.5 and 1.35 kb corresponding to the other DNV. The characterization of the unknown DNV mixed with JcDNV in the larvae of *Ph. operculella* is still in process.

Table I. – Comparison of number and size (in kPb) of JcDNV and PoDNVs restriction fragments of genomic DNAs.

	Bam HI		Bgl II		Hae II	
	JcDNV	PoDNV	JcDNV	PoDNV	JcDNV	PoDNV
A	5.25	5.25	5.85	5.85	5.35	5.35
B	0.31	0.31		4.50		3.30
C	0.29	0.29		1.35		2.05
D					0.50	0.50
E						0.50
	5.85	5.85	5.85	11.70	5.85	11.70

After ten generations of PTM were obtained in the laboratory, we tested the same five concentrations of JcDNV used before on the third generation (2, 4, 6, 8 and 10 ODs). The mortality response increased when compared with the first experiment (Fig. 1B). This increase in the susceptibility of the larvae could be due to the accumulation of some kind of latent viruses.

Activation of the latent viruses by other stressors

As there was an activation status, and in order to establish the hypothesis of a latency phenomenon, it was necessary to find other stressors, free of viruses. We used first over-crowding, already known as a common physical factor affecting latent infections (SMITH 1963). While, we used 1 g of potato per larva in standard condition of the PTM rearing unit, we changed this ratio to be 1 g per 4 larvae. That dramatically decreased the larval survival to 59.7 %. The purification of the dead larvae collected after 14 post-treatment days (second and third instars) revealed the presence of a dominant picorna-like virus with the same previous characteristics, and a low relication rate of a nodavirus resembling the Nodavirus which was previously detected in the cultured cells of the PTM (LÉRY *et al.* 1998), revealed by the presence of a 39 kDa polypeptide after SDS-PAGE analysis (Fig. 2B). There was no evidence for any additional densoviruses.

Another kind of stressor used was EDTA in a chemical intoxication test. There was a correlative effect: 17, 24 and 30 % mortality was observed using 1, 3 and 5 % EDTA, respectively. The dead larvae (second and third instars) were also collected after 14 days. This stress could activate the picorna-like virus and the nodavirus, but there was no evidence for the activation of the densovirus.

These experiments confirmed the activation of mainly two viruses: a dominant picorna-like virus, mainly responsible for most of the mortality response toward the three induction agents used, and a nodavirus activated by non-viral agents only. The densovirus whose complete separation and identification is in process, was only activated by the use of JcDENV. Although, the activation of this densovirus could be established by the JcDENV only, the incidental viral contamination could not be excluded.

Further studies must be directed toward the relationship between the application of PhopGV, already used for the biological control of the PTM, and the activation of these latent viruses, in order to understand the impact of this bioagent and its epidemiology in the environment. The activation of such latent viruses can play an important role in the indirect use of the non-permitted small viruses in biological control programmes while presence of the latent viruses may cause many problems in insect rearing, especially in the mass production of a biological agent, such as the PhopGV. Such a study improves the knowledge in order to control the various aspects of these latent viruses, and can help to provide healthy units for mass production of the bioagents .

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