Increasing the susceptibility of the *Malacosoma neustria* larvae to nucleopolyhedrovirus by a synergistic additive

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Abstract: Additives, such as granuloviruses (GVs) were assessed for potential effects of susceptibility on the host insects to nucleopolyhedroviruses (NPVs). The virulence of a Latvian isolate of *Malacosoma neustria* nucleopolyhedrovirus (MnNPV) was tested. The possibilities to enhance the virus infection by mixing MnNPV with GV were investigated with the following results: larval mortality increased if the CpGV (1x 10^3 capsules/ml) are added. Increases in mortality ranged from 1.09 to 1.24 fold suggesting that some component of the CpGV granule enhanced MnNPV virulence.

Key words: *Malacosoma neustria, nucleopolyhedrovirus, granulovirus, synergistic additives*

Introduction

Several studies were aimed to enhance the infectivity of the insect viruses by mixing the nucleopolyhedroviruses (NPVs) with additives of different origin. A synergistic relationship between a granulovirus (GV) and NPV was first observed in *Pseudaletia unipuncta* by Tanada (1959). Hukuhura et al. (1987) concluded that the degree of enhancement achieved by the synergistic factor varied considerably, depending on the host insect and the virus. Recent investigations showed that the *Trichoplusia ni* GV enhanced, *Spodoptera littoralis* NPV and *Agrotis segetum* NPV infections in their host insects (El-Salamouny et al. 1998).

Researches on biological control with NPV were carried out in the Institute of Biology, University of Latvia, Laboratory of Experimental Entomology since 1986. *Malacosoma neustria* NPV were isolated from collected dead larvae (Jankevica et all., 1998). Biological activity of Mn NPV isolate was characterized by a bioassay using infection with disc method. MnNPV isolate had medium activity (Jankevica, Zarins, 1999).

The aim of the present study was to investigate the effects of *Cydia pomonella* granulovirus (CpGV) on the susceptibility of the European tent caterpillar *Malacosoma neustria* (Lasiocampidae) larvae when challenged by NPV.

Methods

MnNPV isolated in Latvia (Jankevica et al., 1998) was used. GV isolated from *Cydia pomonella* (Zariņš, Eglīte, 1993) were used as an additive to MnNPV. CpGV was multiplied in laboratory culture of *Cydia pomonella.* Dead larvae of *M. neustria* and *C. pomonella* were frozen with liquid nitrogen and homogenised in distilled water with 0.1 % sodium dodecylsulphate (SDS). The homogenate was then filtered through cheese-cloth. Polyhedra and GV capsules were purified by centrifugation on a 30 – 66 % (w/v) linear sucrose gradient. Inclusion bodies bands were collected and washed twice with distilled water. The virus pellet was resuspended in a small volume of deionised water and stored at -18^o C. The virus suspensions in concentrations (1x 10^3, 5x 10^3, 1x 10^4 and 5x 10^4 polyhedra/ml) were used. Sterilised water was used as dillutant. Virus solutions have been evaluated for contaminants, by counting the colonies growing in plate count agar (PCA) after aerobic incubation at 30^{o}C. Solutions where amount of

contaminants were less than 2 x 10^2 were used.

Leafs from apple trees in apple-gardens located in Salaspils were collected. Virus insecticides had not being used in these gardens. Collected leafs were exposed under UV light for 1 h to inactivate bacteria's and viruses. Leaf discs of apple leaves (2 cm in diameter) were prepared using a cork borer, leaf discs were dipped into the virus (NPV and NPV + GV) suspensions with different concentrations, then dried at room temperature. Using fine forceps, the dried discs were transferred, each in a well of the bioassay plate. Single third instar larvae were fed to five discs. After one day the larvae were transferred to fresh, virus free foliage. In control larvae were fed on discs dipped into sterile water. The bioassay treatments were kept under constant conditions, 25^{o}C, 60-70% RH and 16 hour light. Mortality due to infection was recorded daily and up to 15 days. Experiments were repeated 5 times (20 larvae in each replica). Virus activity was expressed as the percentage mortality caused by the NPV. Presence of MnNPV polyhedra in dead larvae was checked by light microscope Amplival. Bacteria were observed in some specimens.

Results

Accumulative mortality of *M. neustria* larvae 15 days following treatments is recorded (Table 1). A 1.09 to 1,24 fold increase in mortality was observed with the addition of GV capsules. These increases were consistently higher in treatments with the highest GV concentrations. Mortality in control was 2.2.%. No typical bacterial infection was observed. Treatment of *M. neustria* larvae with CpGV alone did not cause any mortality.

Table 1.

Effect of *Cydia pomonella* granulovirus as a synergistic factor to *Malacosoma neustria* nucleopolyhedrovirus on the NPV - caused mortality among test larvae.

NPV concentration (polyhedra/ml)	Cumulative NPV –mortality , % (average ± SD)		
	Concentration of GV additive (capsules/ml)		
	0	1x 10^3	1x 10^5
1x 10^3	60.2 ± 2.2	66.8 ± 3.6	68.4 ± 2.7
5x 10^3	69.3 ± 2.4	78.1 ± 2.5	81.1 ± 3.0
1x 10^4	78.2 ± 1.8	94.4 ± 2.2	97.2 ± 2.6
5x 10^4	90.1 ± 1.6	98.3 ± 1.7	98.8 ± 1.0

Discussion

Mortality was enhanced in most instances. Data presented in Table 1 show that, NPV - mortality rate significantly increased if the MnNPV concentrations (5x 10^3 and 1x 10^4 polyhedra/ml) were mixed with CpGV (1x 10^3 capsules/ml). Addition of CpGV (1x 10^3 capsules/ml) to MnNPV (1x 10^4 polyhedra/ml) solution increase mortality of *M. neustria* larvae 1.22 fold. We did not found dead larva with typical GV infection. We determined detectable synergistic effect in the case of combining CpGV with MnNPV. The next step will be investigation of nature of enhancement factor.

Conclusions

A synergistic relationship between a CpGV and MnNPV was observed. The addition of GV enhances the virulence of MnNPV.

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Kopsavilkums

Eksperimentāli tika analizētas *Malacosoma neustria* kodolu poliedrozes vīrusa (MnKPV) efektivitātes palielināšanas iespējas. *Cydia pomonella* granulozes vīrusa (1x 10^3 granulas/ml) pievienošana MnKPV suspensijai palielina *M. neustria* trešā vecuma kāpuru mirstību 1,24 reizes.

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