Virulence of *Malacosoma neustria* nucleopolyhedrovirus Latvian isolates

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Abstract

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The virulence of Latvian isolates of *Malacosoma neustria* multiple nucleopolyhedrovirus (Mn NPV) was tested. To study the possibilities to enhance virus infection we compared virulence and virus production of two *M. neustria* isolates. Studied Mn NPV isolates showed different biological activity.

Key words: *Malacosoma neustria*, nucleopolyhedrovirus, virulence, biological control

Introduction

In nature, nucleopolyhedroviruses (Baculoviridae), cause diseases of insects and regulate their host populations. Nucleopolyhedroviruses (NPVs), are considered to be safe biological insecticides and have a great potential in pest control. The European tent caterpillar *Malacosoma neustria* L. is widely represented in the apple-gardens in south and eastern part of Latvia (Ozols, 1963). Nuclear polyhedrosis viruses have been isolated from several *Malacosoma* species across North America and Europe (Stairs 1964, Schmidt et al., 1994, Keddie, Erlandson, 1995) and from *M. neustria* (Zariņš, Kalniņa 1971, Jankevica et al., 1998) here in Latvia. Mn NPVs have been used successfully against aple tree pest *M. neustria* (Magnoler, 1985, Zariņš, Eglīte, 1993). Mn NPV was used as a basis of 'VIRIN-KSh' that is the virus preparation registered and produced in former Soviet Union. The method of producing the virus formulation was elaborated in Latvia (Zarinsh et all., 1987). It is well known that during storage virus virulence may be changed. Virulence and pathogeneity are important to studies of pathogen - host interactions and may be used in characterization of virus isolates and strains. Determination of time - dose - mortality relationships, along with morphological characteristics, is of importance in determining pathogen isolates which have the greatest potential for controlling target host. The improved bioassay system developed, allows rapid and accurate determination of median lethal dose (LD_50), median lethal time (LT_50), and should be an impetus for further, detailed studies on the biological properties of baculovirus strains (Evans, 1981). The voluminous literature of LD_{50} determined by log-dose probit experiments provides powerful laboratory evidence of

the importance of pathogen population density. The regression lines show that the response (i.e., percentage of infection or mortality) is directly dependent on dosage (i.e., pathogen population density) (Huber, Hughes, 1984). Researchers have demonstrated significant differences in the virulence, host range, and pathology of various strains and isolates of baculoviruses (Gelertner, Frederici, 1986). Infectivity can be enhanced by factors produced by pathogens and by factors present in the environment.

Recently, Laboratory of Experimental Entomology of Institute of Biology, University of Latvia, either has been working to improve methods of biological control of *M. neustria* populations. The present aim was to search for new isolates of NPV in the European tent caterpillar *M. neustria* L. (Lasiocampidae) populations, to determine its virulence and to investigate the possibilities of enhancing virus infection. Quantitative and qualitative aspects of virus production in *M. neustria* larvae were studied.

Materials and Methods

Viruses

Two isolates of *Malacosoma neustria* nucleopolyhedrovirus (Mn NPV) were obtained from infected larvae with nuclear polyhedrosis symptoms. Infected larvae were collected from the well producing apple gardens located in district Saldus, and district Dobele.

Isolation and purification of NPV

Dead larvae were frozen with liquid nitrogen and homogenized in distilled water with 0,1 % sodium dodecyl sulphate (SDS). The homogenate was then filtered through cheese-cloth. The filtrate was centrifuged at 6,000 g for 40 min. Polyhedra were purified by centrifugation on 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 H_2O and stored at -18^{o} C.

Dosage- mortality assays

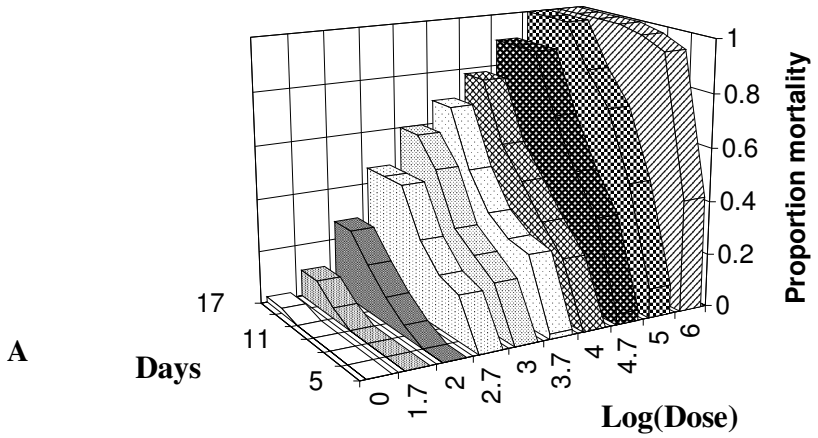
Leaf discs of apple leaves (1cm in diameter) were prepared using a cork borer, measured dose of virus were applied to leaf discs (Evans, 1981), then dried at room temperature. Using fine forceps, the dried discs were transferred, each in a well of the bioassay plate. Single third or fourth instar neonate larva was fed to each disc. After one day larvae were transferred to fresh, virus free foliage. The bioassay treatments were kept under constant conditions, 25 C^{o}, 60-70% RH and 16 hour light, for 16 days. Mortality due to infection was recorded daily and up to 17 days. Experiments were repeated 5 times (20 larvae in each replica). Virus activity was expressed as the percentage mortality caused by the virus (Finney, 1971).

Evaluation of the yield of virus polyhedrae

Third instar *M. neustria* larvae fed on leaf discs with different virus doses were individually homogenised. The polyhedrae count per larva was determined by a haemocytometer.

Results

Biological activity of Mn NPV isolates were characterized by a bioassay. If third instar *M. neustria* larvae were infected, the rate of mortality reached 10%, 27%, 46%, 60%,69%,78%, 90%,100% for the tested doses of $5x10^1$, $1x10^2$, $5x10^2$, $1x10^3$, $5x10^4$ and $1x10^5$ polyhedrae/larva of MnNPV isolate (Saldus), respectively. LD_{50} values determined were 55 ± 10 and 985 ± 19 polyhedral occlusion bodies (polyhedrae) per larva for second and third instar, respectively. The rate of mortality caused by MnNPV isolate (Dobele) reached 10%, 18%, 37%, 40%, 48%, 64%, 82%,98% for the tested doses of $5x10^1$, $1x10^2$, $5x10^2$, $1x10^3$, $5x10^4$ and $1x10^5$ polyhedrae/larva, respectively. LD_{50} values were 80 ± 30 and 3280 ± 380 polyhedral occlusion bodies (polyhedrae) per larva for the second and the third instar, respectively. The relationship between time - dose and mortality for the virus isolates is shown in Figure 1 A,B.



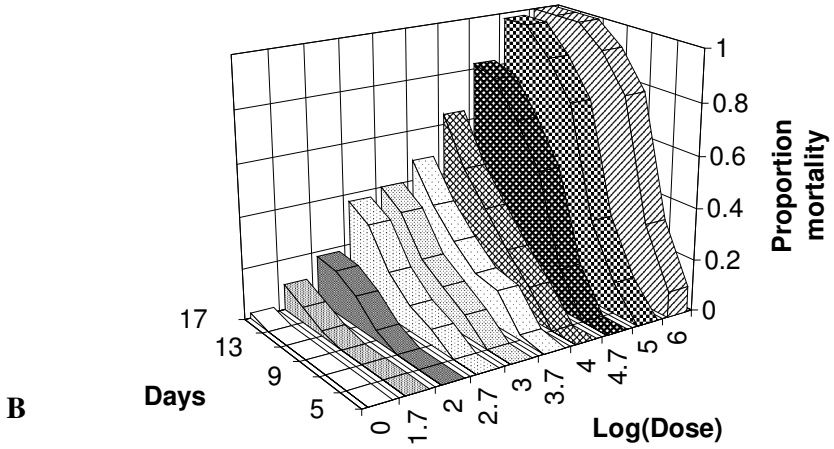


Figure 1. Relationship between cumulative proportion mortality, viral dose, and mortality time of *Malacosoma neustria* third instar larvae. Two isolates were used for bioasays. A-Mn NPV isolate (district Saldus), B - Mn NPV isolate (district Dobele).

The bioasays showed that incubation period of disease was 5 to 10 days after infection with different doses of Mn NPV.

Data in Table 1 show that the amount of NPV polyhedrae produced or virus yield per larva was $2{,}1x10^9$ to $9{,}82x10^9$ viruses/larva after feeding insects on leaves containing different virus doses. Estimated LT_{50} was 6.1 to 14.5 days.

Table 1

Effect of initial virus concentration on LT_{50} and obtained virus yield of Mn NPV in *Malacosoma neustria* 3rd instar larvae

Tested doses	Mortality corrected after Abbot, %	$LT_{50} \pm sLT_{50,}$ days	Day of yield collection	Average yield/larva
$1x10^3$	62,8	$14.5 \pm 0{,}7$	8	$9.82x10^9$
$1x10^4$	78,6	11.9 ± 0.45	8	$3.84x10^9$
$1x10^5$	99	$8.4 \pm 0{,}26$	8	$2.87x10^9$
$1x10^6$	99	6.1 ± 0.17	8	$2.12x10^9$

Addition of plant lecitine (conc.1%) to low doses virus suspension increase larval mortality from 50% to 89%.

Discussion

Morphological characteristics of obtained isolates showed that the dimensions of isolated polyhedra were 850 to 1400 nm. Polyhedrae contained large number of rod-shaped multiple virions (Jankevica et al., 1998). The results of our experiments showed that tested isolates had different virulence. Isolate obtained in district Saldus had higher virus activity and shorter incubation period of disease. The increasing of virus doses caused decrease of the incubation period of disease and the time until insects death.

The comparison of biological properties of both isolates concurs with the DNA analysis (Jankevica and Jankevics, unpublished) that showed differences between isolates.

Results obtained in the experiment performed to evaluate the effect of initial virus concentration on obtained virus yield of Mn NPV in *Malacosoma neustria* 3rd instar larvae does not differ significantly from the conclusions of Rituma and Skujāne (1983), and Im et all. (1989), that to obtain maximum quantity of polyhedrae, it is purposeful to infest third or fourth instar larvae. In the case of using the MnNPV (Saldus) isolate and dose input of $1x10^3$ polyhedrae/larva, the highest yield per larva was obtained with the value of $9.8x10^9$ polyhedrae/larva which decreased to $3.8x10^9$, $2.8x10^9$ and $2.1x10^9$ polyhedra/larva for the

tested concentrations of $1x10^4$, $1x10^5$ and $1x10^6$ polyhedra/larva, respectively. The virus yield was maximal on the 8th day postinoculation.

We conclude that the tested virus isolates showed high virulence and can be used as a source of virus insecticide to control populations of the European tent caterpillar.

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Kopsavilkums

Latvijas Universitātes Bioloģijas institūta Eksperimentālās Entomoloģijas laboratorijā no ievāktu slimu kāpuru materiāla, kas bija ievākts Saldus un Dobeles rajonos, tika izdalīti *Malacosoma neustria* kodolu poliedrozes vīrusa (MnKPV) izolāti. Tika salīdzināta divu izolātu virulence un produktivitāte. Noskaidrotas attiecības starp vīrusa devu, laiku un kāpuru kumulatīvās mirstības proporcionalitāti. Mn KPV (Saldus) izolāts uzrādīja augstāku virulenci.

Veiktie pētījumi ļāva secināt, ka analizētie MnKPV izolāti var tikt izmantoti izstrādājot jaunas vīrusinsekticīda formas.

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