Presence of nucleopolyhedroviruses in natural populations of *Malacosoma neustria* L. (Lepidoptera, Lasiocampidae)

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Abstract: *Malacosoma neustria* nucleopolyhedrovirus (MnNPV) is a potential control agent for European tent caterpillar, *Malacosoma neustria* L. (Lasiocampidae*).* Populations of *M. neustria* were monitored in the eastern part of Latvia as part pf the North Europe Moth Monitoring Program. We developed a PCR-based method to detect polyhedrin-specific MnNPV DNA, in extracts of *M. neustria* larvae and adults. The insects collected from natural habitats were checked for NPV. We first detected MnNPV infection in declining populations in 1997. 1998, when *M. neustria* population densities were low, 63-100% of asymptomatic insects in Liepāja district were NPV-infected.

Key words: *Malacosoma neustria*, population density, nucleopolyhedrovirus, NPV-infection.

Introduction

In nature, nucleopolyhedroviruses (Baculoviridae), are frequently associated with outbreak and declining populations of Lepidoptera. They cause diseases of insects and can reduce population size of their hosts significantly. Nucleopolyhedroviruses (NPVs) are considered to be safe biological insecticides and have a great potential for pest control. Local virus strains and isolates have high activity under the climatical conditions of Latvia (Zariņš, Eglīte, 1993). Polyhedra or proteinaceous occlusion bodies protect the virions from inactivation by environmental factors. The virus also may persist within the host insect population in a latent state (Bilimoria, 1991). Generally natural epizootics caused by NPVs are observed in areas were host populations reach high density. However there is a little information on the persistence and transmission of viruses in pest populations at low densities.

The European tent caterpillar *Malacosoma neustria* L. is often present in the apple-orchards in the southern and eastern parts of Latvia (Ozols, 1963). *M. neustria* prefers members of the family Rosaceae, including apple trees, black cherry, hawthorns and raspberries. Studies of *Malacosoma* populations indicate that like other forest caterpillars they often have 8-11 year population cycles (Myers, 1993). NPVs have been isolated from *Malacosoma* species in Europe (Stairs, 1964, Magnoler, 1985) and here in Latvia (Zariņš, Kalniņa, 1971, Jankevica et all, 1998). Development of laboratory techniques that allow viruses to be identified at low concentrations and in

individuals that are asymptotic is altering our understanding of their role in population cycles.

Recently, at the Laboratory of Experimental Entomology of Institute of Biology, University of Latvia, we have been studying the NPVs persistence and their role in prolonged regulation of insect populations including their occurrence of viral infections in declining populations. The aim of our study was to test *M. neustria* populations for NPVs and to further our knowledge of their occurrence and environmental effects.

Materials and methods

Collecting of insects

Since 1995 monitoring of *M. neustria* population density has been conducted as part of the North Europe Moth Monitoring Program. *M. neustria* adults were collected from natural habitats using light traps. Traps were situated in the eastern part of Latvia. Collections were made at 5 trapping stations belonged to the Latvian Museum of Natural History and were located in the Liepāja district. These stations were identified as Ķoņi, Pape, Grobiņa-1, Grobiņa-2 and Virga. Figure 1 shows average daily temperature per year in the Liepāja district during the period of the study. Light traps were in operation from beginning of June till end September (fig. 1).

Productive apple-orchard (area approximately 20 ha) located in Grobiņa (Liepāja district) was used for monitoring of larval stage in 1996, 1997, and 1998. Virus insecticides have not been used in these regions. Monitoring of larval number was done by counting tents formed by the family groups of *M. neustria* caterpillars. 50% of 3rd or 4th instar larvae from 5 tents were collected. We recorded the level of defoliation in inspected apple-orchards.

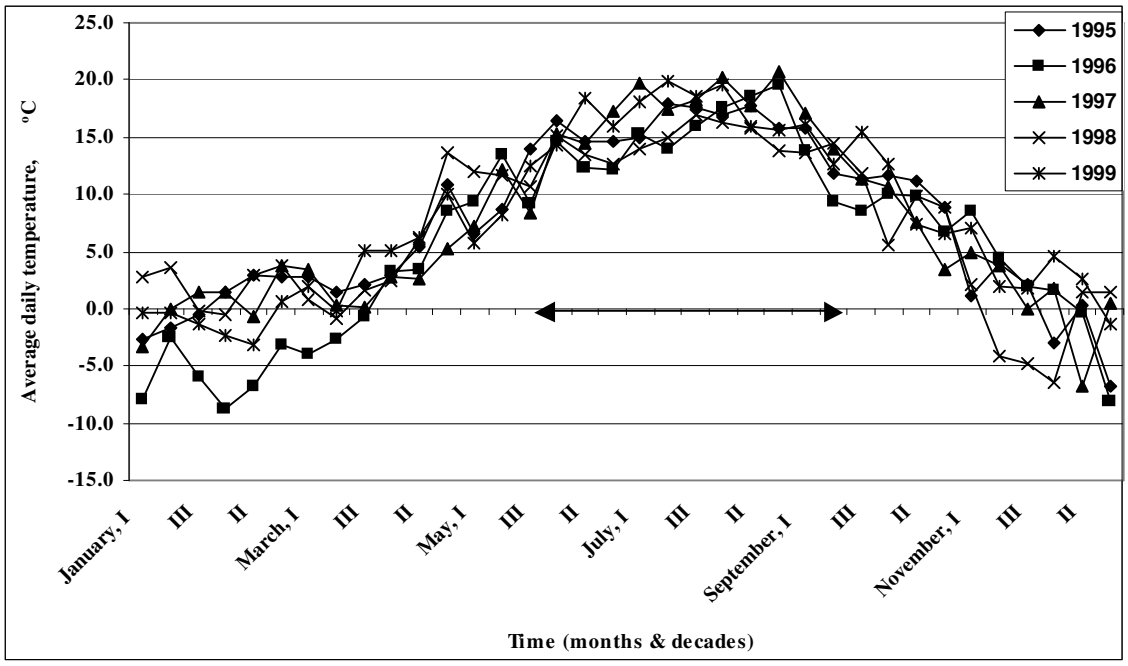

Figure 1. Average daily temperature in Liepāja district from 1995 to 1999 (Report of the Latvian Hydrometeorological Agency, unpublished, with permission). The arrow indicates period when the light traps were in operation.

Determination of presence of NPV in *M. neustria* populations

In 1998, 25% of adults collected from each trapping location were tested for presence of NPV. Part of collected larvae were reared in laboratory on natural food. Percentage of larval survival was calculated. In 1997, 10 % of 3rd or 4th instar larvae collected from each tent were examined for NPV using DNA tests. Collected larvae apparently were healthy with no obvious signs of virus disease. Individuals were catalogued and kept frozen at –18 °C until DNA analysis was made.

Extraction of virus and host genomic DNA from individual insects

Extraction of virus and host genomic DNA from individual insects was done according to Saville et al. (1997). Each adult or larva was homogenised in 600 µl ice-cold TE buffer containing 0.1% SDS using a dunce homogeniser. To 300 µl homogenate, 200 µl 0.1M sodium bicarbonate were added. After 10 min at 37 °C, 200 µl lysis buffer and 2.5 µl RNase A (10mg/ml) were added and incubated for 30 min. Following the addition of 15 µl Proteinase K (10mg/ml), the incubation was continued for a further 30 min. The mixture was extracted once with phenol, twice with phenol: chloroform (1:1) and once with chloroform. The DNA was precipitated with ethanol, washed in 75% ethanol and the air dried pellets were dissolved in 100 µl distilled water (Saville et all., 1997).

Determination and identification of viral DNA by PCR

We used nested PCR to increase the sensitivity of determination. Designed primers correspond to the polyhedrin gene of MnNPV Latvian isolate (Jankevica, 1999). The first set of primers amplified 985 bp, and the second set 708 bp of polyhedrin gene. The PCR reaction mixture contained the following components: insect or viral DNA - 1µl; 10x PCR buffer - 5µl; $MgCl_2$ - 5µl: BSA (1mg/ml) -4µl; dNTP (10mM) - 1µl; Primer1 (10pM/µl) - 2µl; Primer2 (10pM/µl) - 2µl; Taq polymerase 5U/µl (MBI Fermentas) -1µl; water till 50µl. The PCR conditions were: 30 cycles on GeneAmp PCR System 2400 (Perkin Elmer). At the end of the reaction the resulting amplification products were electrophoresed on 1% agarose gel, stained with ethidium bromide and visualised under short wave UV light. The size of DNA fragment was calculated.

Results

Our observations showed that in 1995 *M. neustria* was widespread in the Liepāja district. We collected *M. neustria* at all trapping sites, Ķoņi, Pape, Grobiņa-1, Grobiņa-2 and Virga (Table 1). *M. neustria* populations in Liepāja district had the highest density in 1995. Overall the largest population was observed in Virga; 1154 adults were collected. The total amount of collected adults in the Liepāja district decreased significantly during the next four years. The density of *M neustria* population (Virga) was diminished 38 times during monitoring. In Ķoņi and Grobiņa-2 populations fluctuated. Adults had no obvious signs of disease. We did not find any highly defoliated areas in the Liepāja district. In 1999, populations were very low. We caught only 12 adults at one site Virga.

Monitoring of *M. neustria* populations was difficult in egg and larval stages, because the population density during the period (1996 - 1998) was low. Our

observations of the *M. neustria* life-cycle showed that the eggs (150 to 250) are laid as a mass that encircles a twig of tree. A few weeks after eggs are laid (August, September), the young caterpillars are fully formed within them. Hatching takes place about the time when first new leaves appear in the following spring near the end of April. Caterpillars from one egg mass form a tent in and on which they live. The colonial habits of tent caterpillars enhance monitoring and measurements of larval survival. Our observations in study plot Grobiņa (1996) showed that the density of *M. neustria* population was low (<1 nest on 10 trees). Larval mortality in population was 15%. Defoliation of trees was low, not more than 10%. In 1997, only 6 nests were located in a study plot in Grobiņa. Development of cocoons occurred in the middle of June. Adult moths flew from the beginning of July till September (figs. 2, 3). Most of the flying adults were trapped during the period from the 9th July till 14th August in 1995, 1997 and 1998. While in 1996, adults started to fly two weeks later.

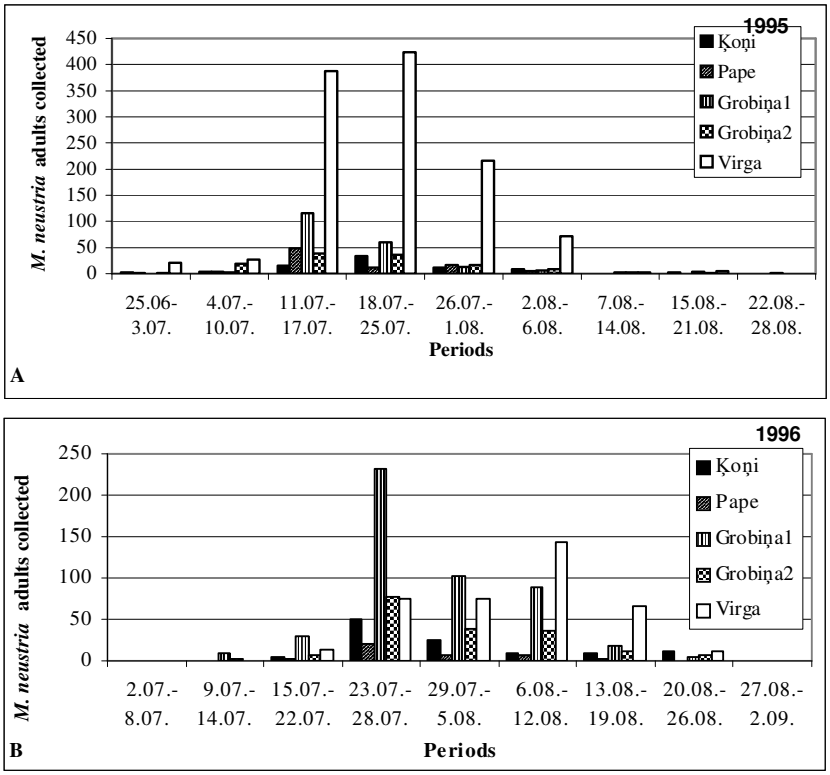

Figure 2. Seasonal dynamics of *Malacosoma neustria* adult population determined by light trapping in 5 sites in the Liepāja district. A - 1995, B - 1996.

We used a PCR-based method to detect polyhedrin-specific MnNPV DNA in the extracts of *M. neustria* larvae or adults. Laboratory experiments showed that optimised method could detect MnNPV infection of larvae 2 days after *per os* infection (Jankevica, 1999). Table 2 shows that MnNPV DNA was detect in all populations examined. For example, 63% of examined *M. neustria* larvae collected in Grobiņa in 1997 were infected by NPVs. In 1998 we detected MnNPV in 75-100% of *M. neustria* adults collected in the Liepāja district. Reults showed that collected *M. neustria* larvae and imago had latent or sublethal NPV-infection. Figure 4 illustrate the results obtained from testing DNA extracted from *M. neustria* larvae or adults collected in different sites.

Discussion

We observed the decrease of the *M. neustria* population density in 1996 in the Liepāja district. Our observations agree with the conclusion of Morris long-term study (1963); caterpillar populations declined before food became limiting, since we did not record high defoliation in this area. Mortality and the progression of mortality through life stages of *M. neustria* might be caused by different agents. We had methodological problems with life table studies, because obtaining an accurate count of insects and the mortality agents, particularly when population density is low, is very difficult. As population limiting factors we can denominate influence of weather, effects of parasitoids and pathogens. In literature, the following conditions are reported to favour outbreaks of forest tent caterpillars: a warm spring, early spring followed by cooler weather, a cool fall, cold winter and warm spring (Martinat, 1987). Recent studies doubt on the climate and weather hypotheses. During the 40 year observations in Canada, no correspondence between increase in defoliation and warm spring conditions were found (Myers, 1993). Inclement early spring weather or poor food quality might be expected to have the greatest influence on the survival of young larvae. However, we did not observe any significant influence of temperature on population density, therefore our observations concurred with conclusions of J. Myers (1993) that weather influence is an unlikely explanation for fluctuation of population density. Our investigations showed that seasonal dynamics into population might be influenced by outdoor temperature. In 1996, after a cold winter and cold spring flying of adults started two weeks later than in 1995 and 1998. Our observations show that for several years after population peak, the survival of *M. neustria* continues to be low. In literature we found reports, that unfavourable spring weather combined with virus disease produce cyclic abundance of tent caterpillars (Myers, 1993). Viral diseases have received renewed attention now that the molecular techniques allow viral DNA to be detected at low concentrations both in the environment and in asymptomatic insects. Using a sensitive PCR-based method and primers described by Jankevica (1999), NPVs were found in different stages of insect's development: adults, larvae and cocoons. NPVs increased the mortality of insects in different stages of their development and diminished the pest population density. The presence of viruses in larval and adult stages may be caused by a latent form of virus disease or sublethal effects in cases where the caterpillars ingest virus shortly before they pupate and do not die but instead become smaller, less vigorous adults. Determined high presence of virus in *M. neustria* populations (63-100%) conquer with conclusion of Y. Myers (1993) that viral disease is the elusive driving force in cyclic insect populations. Our observations showed that viruses persisted in *M. neustria* populations and caused mortality of insects in different stages of their development and diminished the pest population density.

Conclusions

Observations shoved that declined populations of *M. neustria* had latent or sublethal NPV infection.

The method for the detection of polyhedrin-specific DNA sequences using PCR has been proved to have good possibilities for the determination of presence of viruses in pest populations.

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Kopsavilkums

Malacosoma neustria kodolu poliedrozes vīrusu (Mn KPV) ir uzskatāms par perspektīvu augļu dārzu kaitēkļa ābeļu vērpēja *Malacosoma neustria* L. (Lepidoptera, Lasiocampidae) bioloģiskās kontroles aģentu. No 1995. līdz 1999. gadam Latvijā, projekta "Ziemeļeiropas Tauriņu monitoringa programma" ietvaros, tika veikts *M. neustria* populāciju monitorings. *M. neustria* sastopamība 1995. gadā bija vislielākā, turpmākajos 4 gados tā būtiski samazinājās. Būtiska sakarība starp klimatiskajiem apstākļiem un *M. neustria* sastopamību netika konstatēta. LU Bioloģijas institūtā, tika izveidota jutīga, uz PCR balstīta, metode poliedrīn-specifiskas Mn KPV DNS noteikšanai *M. neustria* kāpuros un pieaugušos īpatņos. Ābeļu vērpēji, ievākti to dabiskajās dzīvotnēs Liepājas rajonā, tika pārbaudīti uz Mn KPV DNS klātbūtni. Pielietojot jauno diagnostikas metodi *M. neustria* populācijās tika konstatēta KPV infekcija. 1998. gadā, kad *M. neustria* sastopamība bija ļoti zema, 63-100% no Liepājas rajonā ievāktajiem un pārbaudītajiem ābeļu vērpējiem tika konstatēti KPV. Veiktie pētījumi ļāva secināt, ka izveidotā metode poliedrīn-specifiskas Mn KPV DNS noteikšanai, var tikt izmantota vīrusa sastopamības noteikšanai kaitēkļu populācijās.

References

Bilimoria S. 1991. The Biology of Nuclear Polyhedrosis Viruses. – In: Kurstak E. (ed.). Viruses of Invertebrates. Marcel Dekker, New York: 1-72.

Jankevica L., Čudare Z., Ose V. 1998. New isolate of *Malacosoma neustria* nuclear polyhedrosis virus in Latvia. - J. Invertebr. Pathol., 71, 3: 283-285.

Jankevica L. 1999. Ecological interactions between baculoviruses and pest populations and their role in biological control. PhD Theses, University of Latvia, Riga: 1-50.

Magnoler A. 1985. [Field evaluation of Baculovirus against *Malacosoma neustria* L. in Sardinia]. - La difesa delle piante, 2: 329-338 (in Italian).

Martinat P.J. 1987. The role of climatic variation and wather in forest insect outbreaks. - In: Barbarosa P., Scultz J. (eds). Insect Outbreaks. Academic Press, New York: 241-268.

Morris R.F. 1963. The dynamics of epidemic spruce budworm populations. – Mem. Entomol. Soc. Canada, 31: 1–332.

Myers J.H. 1993. Population outbreaks of forest Lepidoptera. – Amer. Scientist, 81: 240-251.

Ozols E. 1963. Lauksaimniecības entomoloģija. Rīga: 1-510.

Saville G.P., Huges D.S., Shreeve T., King L.A. and Possee R.D. 1997. Baculovirus insecticides: detection of latent baculoviruses in natural insect populations. - In: Proc. Internat. Symp. "Microbial Insecticides: Novelty or Necessity?". UK: 255-260.

Stairs G. 1964. Infection of *Malacosoma disstria* Hubner with nuclear polyhedrosis viruses from other species of *Malacosoma* (Lepidoptera, Lasiocampidae). - J. Insect Pathol., 6: 164-169.

Zariņš I., Eglīte G. 1993. Investigation of entomopathogenous viruses in Latvia and their potential as pest control agents. – Proc. Latvian Acad. Sci., Section B, 57: 49-53.

Zariņš I., Kalniņa L. (Зариньш И, Калниня Л.) 1971. [Some aspects on activation of latent infection of Nuclear polyhedrosis of the European tent caterpillar - *Malacosoma neustria* L.]. - In: Viruses of plants and insects. Latvian Agricultural Academy, Jelgava: 42-48 (in Russian).

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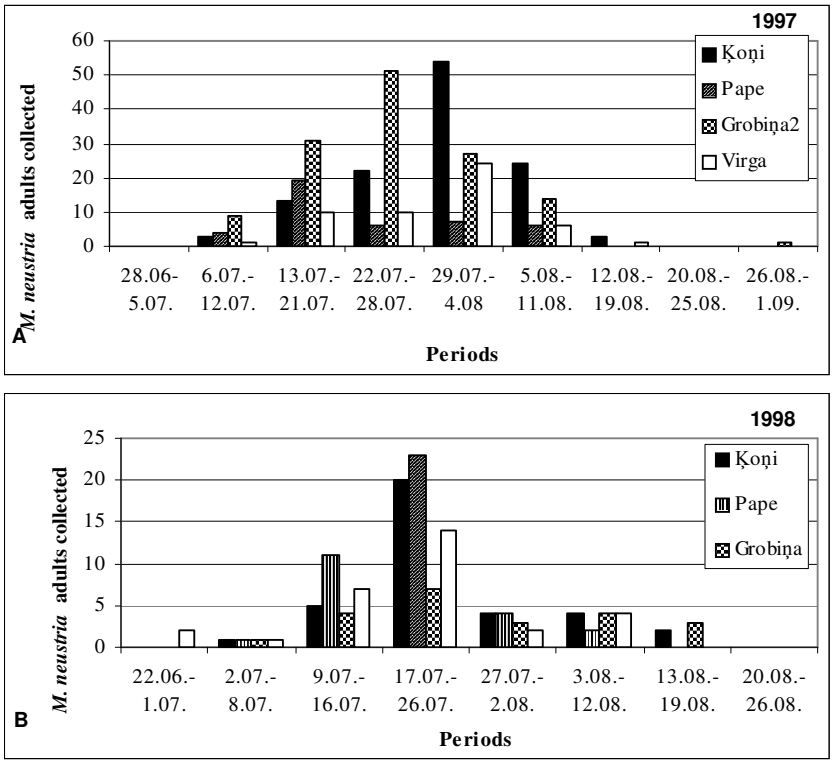

Figure 3. Seasonal dynamics of *Malacosoma neustria* adult population determined by light traping in 4 sites in the Liepāja district. A - 1997, B - 1998.

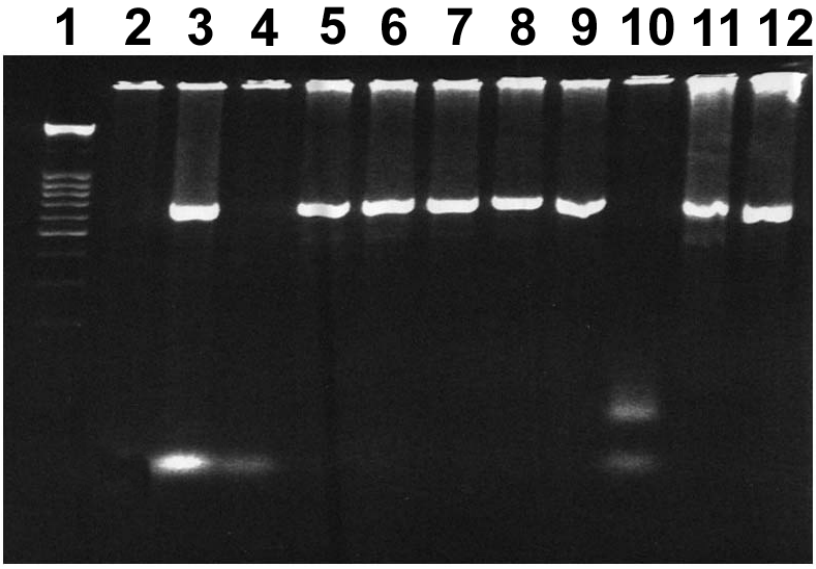

Figure 4. PCR analysis of DNA samples extracted from individual insects collected at different sites. Electrophoresis of PCR products in 1% agarose gel. The arrow indicates PCR product. *M. neustria* larvae (lane 3,4,5) and adults (lane 6,7,8) collected in Grobiņa, *M. neustria* adults (lane 9,10,11) collected in Virga. 100 bp ladder (lane 1), negative control - distilled water (lane 2), positive control - plasmid containing polyhedrine gene (lane 12).

Table 1. Number of *Malacosoma neustria* adults collected in Liepāja district using light traps from 1995 to 1999.

Trapping site	Amount of collected insects per year				
	1995	1996	1997	1998	1999
Ķoņi	76	112	119	36	0
Pape	86	39	42	41	0
Grobiņa-1	202	483	-	-	-
Grobiņa-2	121	179	133	22	0
Virga	1154	385	52	30	12
Total	1638	1198	346	129	12

- mean not done

Table 2. Detection of MnNPV in *Malacosoma neustria* adults and larvae.

Trapping site or study plot	Year of collecting	Developmental stage of insect	Number of examined insects	Number of NPV-infected insects	% NPV positive
Grobiņa	1997	larvae	30	19	63
Grobiņa	1998	adults	6	6	100
Pape	1998	adults	11	10	91
Virga	1998	adults	8	6	75