Biological control of the European tent caterpillar *Malacosoma neustria* L. (Lepidoptera, Lasiocampidae) population with different virus formulations

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Abstract

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The Latvian isolate of *Malacosoma neustria* nuclear polyhedrosis virus (Mn NPV) was used as a source for the production of virus insecticide. In recent years novel virus insecticide formulations have been developed and tested using environmentally - friendly matrix materials. The persistence of NPV in the agroecosystems was measured and the adhesion of virus formulations to apple leaves was determined. Lysine KKL, polyglucine, a by-product of citric acid production and molasses of peat used as additives, increased the retaining of viral polyhedrae on apple leaves after artificial rainfall for 30%. The mean percentage of larval mortality caused by the virus formulations with new additives after exposing in artificially simulated rain was 0.5 to 3 times higher than that in the control.

Key words: *Malacosoma neustria*, nuclear polyhedrosis virus, biological control

Introduction

Baculoviruses including nuclear polyhedrosis viruses (NPVs) are considered to be safe bioinsecticides and therefore have great potential in integrated pest control. NPVs have been formulated and applied as biological insecticides against pest populations (Huber, 1986). Biopreparations based on NPVs may be considered for the following reasons: 1) high host-specificity, no evidence of occurrence in non-arthropod hosts and rest of benefical insects (GrÖner, 1986); 2) distribution and multiplication of viruses in pest populations can control the pest population for several years after initial spraying (Bird, 1961, Young, Yearin 1982), 3) local virus strains and isolates have high activity in the climatic conditions of Latvia (Zariņš, Eglīte, 1993). The nuclear polyhedrosis viruses have different capabilities of surviving in abiotic environment. NPVs described are inactivated by different environmental factors such as sunlight, high summer temperature, humidity and rainfall. The survival capacity of NPV is influenced by chemical degradation in the water, soil or on plant surface. Rain is an important factor of the dispersal of viruses on trees, such as viruses of the tussock moths (Thompson, 1978) and sawflies (Cunningham, Entwistle, 1981). Thompson (1978) reported that in one case the percentage of contaminated current years foliage increased from 12% on the day before a light rain to 100% two days after the rain, and the percentage of virus-infected larvae increased from 23 to 99% during this period. Most studies of rainfall and virus transmission are concerned with artificial virus formulations. The inactivation rate of the viruses may be slowed down using various additives to the virus preparations. The formulated virus preparations are more or less easily washed out from foliage depending on the formulation used (Cunningham, Entwistle, 1981, Mohamed et all, 1982). The role of rainfall in naturally occurring epizootics has not been well documented. Because UV light is known to inactivate insect viruses (Ignoffo et all, 1977, Jacques, 1977) rainfall could do more than simply move virus and it can help to prolong the period of activity by bringing it to places protected from inactivation factors.

The aim of our studies was to test the new virus insecticide formulations developed in Laboratory of Experimental Entomology of Institute of Biology, University of Latvia, with good physical characteristics and use the preparations for biological control of the European tent caterpillar. We compared the persistence of polyhedra and Mn NPV activity in different formulations after artificial rainfall.

Material and methods

Mn NPV isolated in Latvia was used as the basis of a virus insecticide. Polyglucine, molasses of peat, lysine KKL, the by-product of citric acid production was used as additives in the formulations and tested. Formulations containing bentonite were used as positive control.

Suitability of the additives used in the virus formulations was examined in field trials from 1990 to 1995. The efficiency of the virus preparation was expressed as the percentage mortality caused by the virus using the method of Abbott (Abbott, 1925). The experiment was performed to study the influence of artificial rainfall on the persistence of viral polyhedra after application of virus formulation that contains different additives.

Virus application and artificial rainfall test

Apple-tree branches were sprayed with virus preparations ($2x10^7$ polyhedra/ml) using a hand sprayer, concentration of tested additives was 2%. When foliage had dried, simulated rain was applied to apple tree branches. Air-blast sprayer was used as water pump. The spray head was held of 2m distance from the branches. Rain was applied to branches for 10 and 30 minutes. By measuring a rain gauge under the branches we determined that these applications were be equivalent to 20 mm, 100 mm and 300 mm of naturally rainfall, respectively.

Bioassay and DNA-DNA hybridization

After simulated rain application branches were dried and leaves were randomly collected for bioassays and 200 discs (10 mm diameter) were cut out for DNA-DNA hybridization.

Persistence of virus viability after artificial rainfall was tested using a bioassay. Third instar larvae of *M. neustria* reared on natural food in special cages (0.5 x 0.35 x 0.35 m) were fed on sprayed and exposed to the simulated rain leaves for one day. Experiments were repeated 4 times, 30 larvae in each replica.

A specific- DNA-DNA hybridization assay (Ward et all, 1987, Kukan, Meyer, 1995) was used to detect virus on the foliage after the simulated rain. The amount of the persisted polyhedrae was determined. 200 leaf discs were washed and analyzed for each sample with DNA- DNA hybridization. The experiments were repeated 5 times, 40 discs in each replica. We used ^{32}P labeled DNA probe capable to detect MnNPV produced in the Institute of Microbiology and Virology (Sharipo, 1991) and a modified method of DNA-DNA hybridization (Vilnis et al., 1990, Sharipo, 1991) for the detection of MnNPV in the environment.

Results

Field trials with the new virus insecticide formulations indicated that high levels of mortality (89-96%) of second and third instar larvae could be achieved 10 days after the spraying. Table 1 shows the amount of Mn NPV polyhedrae on sprayed apple tree leaves after exposing them in artificial rain. Loss of the polyhedrae on apple-tree leaves after simulated rain was 70-93% in variants with additives and 99% in the control (virus-water suspension without additives).

Table 1

Amount of Mn NPV polyhedrae on apple-tree leaves after artificial rain containing different additives.

Additive in the virus preparation	Amount of polyhedrae on 1 cm^2 leaf surface depending on precipitation		
	0 mm	20 mm	100 mm
Polyglucine	20000	1250	620
Molasses of peat	19000	4400	225
Lysine KKL	20000	6200	620
By-product of citric acid production	18000	3200	200
Bentonite	12000	1250	175
Control - virus-water suspension	19000	300	20

The bioassay shows that the larvae fed on the leaves exposed to the artificially simulated rain died after 10-15 days. Efficiency of virus preparations containing different additives was 90 to 96% before and 76 to 84% after exposing the leaves to the artificial rain equal to 20 mm (Figure 1). After exposing the branches for 30 min to artificial rain equal to precipitation

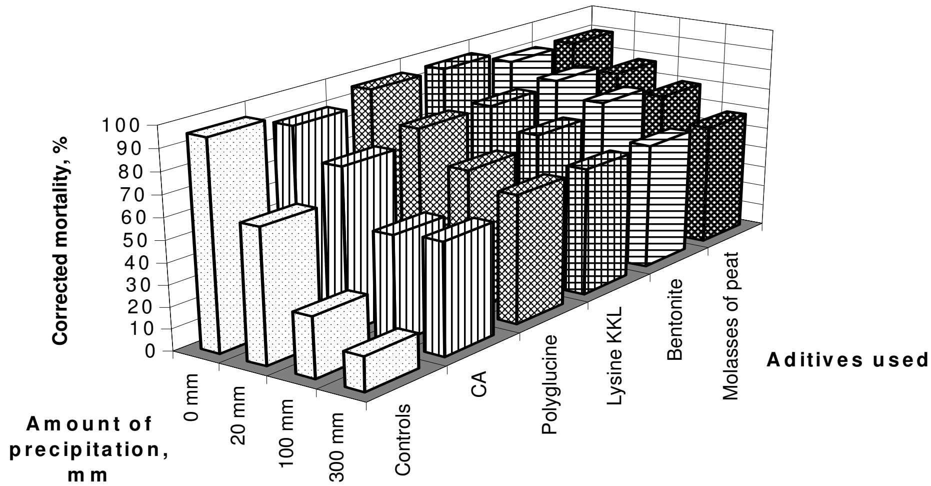

Figure 1. *Malacosoma neustria* larval mortality caused by different virus preparations after exposing to artificial rain. 300 mm the efficiency of the preparations containing additives was 52 to 60%, but in the control without additives - only 18%.

Discussion

The Latvian isolate of *Malacosoma neustria* nuclear polyhedrosis virus (Mn NPV) is a active and selective agent for the control of the European tent caterpillar. All of the tested additives gave good wettability of dispersible dry formulations (Jankevica, Zarins, 1997) as well as promoted adhesion to the plants.

The persistence, distribution and accumulation of NPVs after artificial rainfall were determined to optimise successful biological control of the *M. neustria* population. Results obtained in the experiment performed to evaluate the influence of rainfall on the persistence of virus activity after the application of virus formulation does not differ significantly from the conclusions of Cunningham & Entwistle (Cunningham, Entwistle,1981) that additives reduce the influence of rainfall.

Used additives lysine KKL, the by-product of citric acid production, molasses of peat, polyglucine and Bentonite increased the persistence of the polyhedrae 20, 10, 13, 3 and 3 times, respectively in comparison with control. Results showed that directly after spraying the new tested additives increased adhesion of the virus polyhedrae to apple-tree leaves approximately 2 times in comparison with Bentonite. The used additives increased the efficiency and provided the persistence of activity of the virus preparations. The mortality of *M. neustria* larvae fed on the leaves exposed to artificial rain did not show significant differences among the preparations with additives lysine KKL, polyglucine, and molasses of peat. The effectivity of the preparations with the by-product of citric acid production is lower.

Application of additives exert the influence of rainfall. The amount of polyhedra more than 200 polyhedrae on $1cm^2$ leaf surface exceeds LD_{50} for 2nd instar *M. neustria* larvae and is enough to infect insects in population. The 3rd and 4th instar larvae with high feeding activity and LD_{50} of 1200 polyhedrae (Zariņš, Eglīte 1994), got a sufficient dose of viruses by eating up 5-10 cm^2 of the leaves which had been exposed to artificial rain.

Obtained results concur with the conclusion that the new virus insecticide formulations secure the persistence of virus activity 22 days after spraying and the lysine KKL, polyglucine increased the persistence of the polyhedrae and virus activity (Jankevica, Zarins, 1997).

Conclusions

The new virus insecticide formulations can be used to control populations of the European tent caterpillar. The new additives used in the preparation of the virus insecticides strongly reduce the harmful effect of weather conditions on the persistence of viral activity.

Tested additives of virus preparations retain virus persistence on plants after the rain.

The best efficiency was shown by the formulations with lysine KKL and polyglucine. The used method of DNA-DNA hybridization has been shown to have good possibilities for the evaluation of the amount of virus polyhedrae on leaf surface and shows no significant difference with the results obtained by the bioassay.

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Kopsavilkums

Ābeļu vērpēja *Malacosoma neustria* kodolu poliedrozes vīrusa (MnKPV) Latvijas izolāts tika izmantots vīrusinsekticīda izveidošanai. Latvijas Universitātes Bioloģijas institūta Eksperimentālās Entomoloģijas laboratorijā tika veidotas un izvērtētas jaunas preparatīvās formas, izmantojot videi draudzīgas pildvielas. Darba mērķis bija noteikt pildvielu pieliptspēju, saglabāšanos ekosistēmā un to spēju pasargāt vīrusus no noskalošanās lietū. Lizīns KKL, poliglukīns, kūdras melase un citronskābes ražošanas blakusprodukts par 30% palielina MnKPV poliedru saglabāšanos uz ābeļu lapām. Izmantojot biotestu noskaidrots, ka vīrusa aktivitāte un izsauktā kāpuru mirstība, pielietojot jaunās pildvielas, pēc eksponēšanas lietū ir no 0,5 līdz 3 reizes lielāka kā kontrolē, kur pildvielas netika lietotas.

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