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EFFECTS OF VIRAL EPIZOOTIC INDUCTION IN POPULATION OF THE SATIN MOTH LEUCOMA SALICIS L. (*LEPIDOPTERA: LYMANTRIIDAE*)

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Abstract: An epizootic was induced by introduction of *Leucoma salicis* nucleopolyhedrovirus (LesaMNPV) into satin moth (*L. salicis*) population feeding on poplar *Populus nigra* L. Introduction of LesaMNPV virus at a rate 4×10^2 of inclusion bodies per tree into insect population (stage L_3 and L_4) resulted in a rapid outbreak collapse both in the peak and early decline phases. A rate of epizootic development depended on healthiness of satin moth larvae before the treatment. Insect population with a high level of nucleopolyhedrovirus (app. 21% and 26% infected insects) reached the epizootic peak on the 18^{th} day after the treatment (85% and 86% infected insects). Induced epizootic resulted in the decline phase of *L. salicis* population that continued for subsequent 6 years. Accidental occurrence of fungus *Beauveria bassiana* (Bals.) Vuill. extended the population decline phase for the period of at least 8 years. Induced epizootic caused a rapid collapse of satin moth outbreak with direct transition from the population peak phase into the population collapse phase with omission of the decline phase. This pattern was not observed in not treated populations.

Artificially induced epizootics affected healthiness of insect pupae and adults as well the reproductive potential of females and healthiness of offspring. The weight of pupae obtained from treated larvae was lower when compared with the control. Virus infections were found more frequently in female pupae than in male pupae. This resulted in a 6–28 fold decrease of the number of egg masses and 3.5–5 times lower numbers of eggs deposited by females. Offspring of infected pairs showed symptoms of viral infection (15–28% infected larvae) and the number of offspring was 70–800 times lower in comparison with offspring of not infected pairs.

This study presents the results of induced epizootic and has been the first attempt to review and sum up results of long-term research on evaluation of nucleopolyhedrovirus efficacy in natural conditions.

Key words: *Leucoma salicis* L., nucleopolyhedrovirus (LesaMNPV), *L. salicis* cypovirus, *Beauveria bassiana* (Bals.) Vuill., induced epizootic

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INTRODUCTION

The satin moth *Leucoma salicis* L., has been reported for many years as an important insect pest of poplar trees growing in city parks, urban surroundings and along roads. The pest has become a serious threat for its environment mainly due to the lack of efficient biological methods of its control.

The first reported method of satin moth control was mechanical destruction that was possible only in low plantations and tree plantings (Schnaiderowa 1959). Control strategies were changed along with availability of chemical insecticides on the market. Initially, these were contact insecticides DDT and HCH. Less drastic preparations for insect control replaced these chemicals after finding that chemical pest control destroyed beneficial fauna and posed a threat for humans. Most often applied control means for the satin moth were based on *Bacillus thuringiesis*. These showed high efficacy in Romania (Petcu *et al.* 1974), The Netherlands (Schotveld and Wigbels 1975), Austria (Donaubauer 1976), Georgia (Tsilosani *et al.* 1976), Bulgaria (Kuzmanova *et al.* 1980), Switzerland (Maksymov 1980) and Hungary (Szalay-Marzso *et al.* 1981).

Although a role of nucleopolyhedrovirus LesaMNPV in regulation of the numbers of satin moth populations was recognized the virus was rarely used for control of this pest. Field tests with the use of NPV virus were carried out in former Yugoslavia (Sidor 1967), efficacy of NPV virus was compared with efficacy of *B. thuringiensis* in Belgium (Nef 1975), and in The Netherlands (Grijpma *et al.* 1986) it was proposed to combine NPV virus with pheromones in open traps.

Environment friendly and safe for humans biopreparations based on nucleopolyhedroviruses of herbivorous insect pests have been known for many years. They have been used for efficient insect pest control and protection of crops in agriculture, horticulture, orchards and forests (Hunter-Fujita *et al.* 1998).

The presented study on virus-induced epizootic in natural outbreak population of *L. salicis* and its effects was instigated by encouraging results of laboratory tests and field observations (Ziemnicka 1981), and also by a need to fill gaps in scarce information on a role of LesaMNPV in regulation of natural population numbers of the satin moth constantly damaging poplar stands.

MATERIALS AND METHODS

Treatment site

The treatment for virus induced epizootic was carried out on the outbreak population of the satin moth feeding on approximately 20-year-old poplars *Populus nigra* L. growing along the road Pobiedziska-Gniezno, 19 km away from Poznań, Wielkopolska (population „Pobiedziska”). The poplars (120 trees) were divided into 4 sectors (I–IV), 30 trees each (Fig. 1). Sector I was situated at the beginning of the experimental area and Sector III separated the experimental sectors (II and IV) from each other. Sectors II and IV were divided into strips with 12, 6 and 12 poplar trees. Within each strip first 12 trees were treated with virus suspension and next 12 trees remained untreated control. The 6 trees in between were treated as neutral zone separating treated trees from untreated ones.

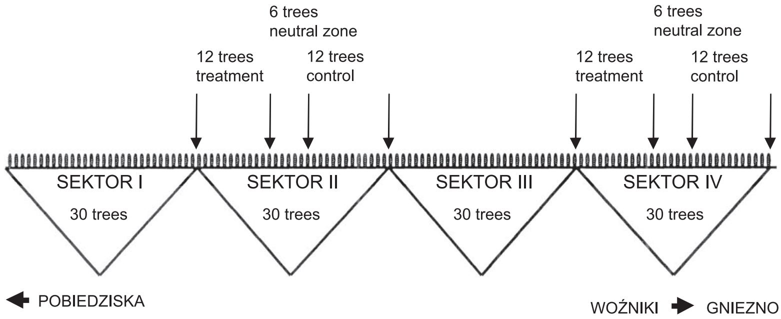

Fig. 1. Diagram on the experimental site of induced epizootic in satin moth population "Pobiedziska"

Preparation of virus suspension and treatment

Virus applications were carried out with the use of LesaMNPV powder virus formulation obtained following the semi-technical methods described by Ziemnicka (2007). After preparation of basic suspension consisting of 1 g of virus powder (1.0 x 10^{12} inclusion bodies) in 1 liter of distilled water with addition of adjuvant Sandovit (0.001%), spray liquid was prepared which consisted of 200 ml of virus basic suspension and 10 l of water. For 50 l of spray liquid 1 g of powder virus formulation was used. Each 10-liter load was enough for spraying 5 poplar trees and on average 4 x 10^{10} inclusion bodies were applied per one experimental tree. The treatment was applied from a crane for construction works at height and with the use of knapsack sprayer.

Collection of insects for diagnostics

Satin moths were collected during the last decade of May (spring-summer generation) for next 10 years after the treatment (1981–1990) and also after 7-year break (1991–1997) until 2007.

In the first year of observations the insects for diagnostic tests were collected 3 times on day 0, 18 and 34 after the treatment. During subsequent years the insects were collected once during the last decade of May.

Larvae and pupae of *L. salicis* were collected from tree trunks (at height up to 2 meters) of treated and control trees, counted and carried to the laboratory. Next, pupae were sexed, weighed and diagnosed. The pupae were maintained in 10 liter glass jars until adults emerged and eggs were laid. The adults were diagnosed, and egg masses were counted and then incubated. Hatched larvae were bred on poplar (*P. nigra*) leaves in laboratory conditions and larvae at the L_2 stage were diagnosed for occurrence of virus and other pathogens with the use of microscopic techniques.

Evaluation of treatment efficacy

The treatment efficacy was evaluated in the first and subsequent years of observation based on the number of larvae collected from treated trees in comparison to the number of larvae collected from control trees. The population parameters such as its healthiness, including that of offspring, dynamics and reproductive potential were compared. Collected dead and alive insects were diagnosed with the use of microscope following microscopic techniques used in insect pathology (Ziemnicka

1981). The reproductive potential was evaluated based on the number of egg masses deposited by females and the number of eggs in egg masses.

RESULTS

The virus epizootics was induced in larvae of *L. salicis* in the stages L_3 and L_4 on 28 May, 1981. The observations of virosis expansion and the treatment efficacy were carried out for 15 years.

Before the treatment (day 0) satin moth population was probably in the phase of decline (Fig. 2, 4). In Sectors II and IV, the number of larvae on tree trunks at a height 2 m from the ground amounted per tree 18–26 and 29–39, respectively. The epizootic was induced into satin moth population with dominant nucleopolyhedrosis (21% infected larvae in Sector II and 26% infected larvae in Sector IV) (Fig. 6, 7).

Additional introduction of NPV virus into infected population resulted in a rapid increase of virus infections in insects in both Sectors. On the 18^{th} day after the treatment the number of insects with virosis increased to 86% in Sector II and 85% in Sector IV (Fig. 6, 7). This beneficial epizootic effect was indicated by 4 times higher number of virus infections in Sector II and 3 times higher in Sector IV when compared with the level of infection on the day 0 of observation. On the 34^{th} day after the treatment the percentage of virus infected larvae was decreased to 48.3% in Sector II and 36.5% in Sector IV. Expansion of virosis in the control part of Sector II on days 0, 18^{th} and 34^{th} was decreasing and amounted for 23%, 20% and 15%, respectively while in the control part of Sector IV it was increasing and amounted 7%, 11% and 23%, respectively.

The effect of induced epizootic was more visible in pupae and less in adults (Table 1). Virosis was more often diagnosed in female pupae (40%) than in male pupae (8–16%). In adults, independently of their sex the virus was diagnosed sporadically (3.3%–4.0% infected insects). Adults obtained from control trees were healthy. The reproductive potential of insects treated with the virus was lower when compared with control insects (Table 2). On the whole, for the two experimental sectors this was indicated by app. 3.5 times lower number of egg masses and 6 times lower number of eggs per one female as well as 800 times lower number of offspring when compared with control insects. Approximately, 29% of hatched larvae died due to virus infection before entering diapause (this result is fairly accurate due to a low number of hatching larvae).

Table 1. Healthiness of pupae and adults of the satin moth (*L. salicis*) collected from untreated (control) poplar trees and treated with nucleopolyhedrovirus LesaMNPV (Pobiedziska 1981)

Stage	Sex	Number of collected insects				% of insects with virosis			
		Sector II*		Sector IV**		Sector II		Sector IV	
		control	treated	control	treated	control	treated	control	treated
pupa	female	54	51	84	37	12.0	40.0	3.3	39.6
	male	30	27	52	35	0.0	8.0	3.3	16.5
adult	female	28	25	28	24	0.0	4.0	0.0	3.3
	male	34	25	31	30	0.0	4.0	0.0	3.3

* diagnostic sample consisted of 25 larvae
** diagnostic sample consisted of 30 larvae

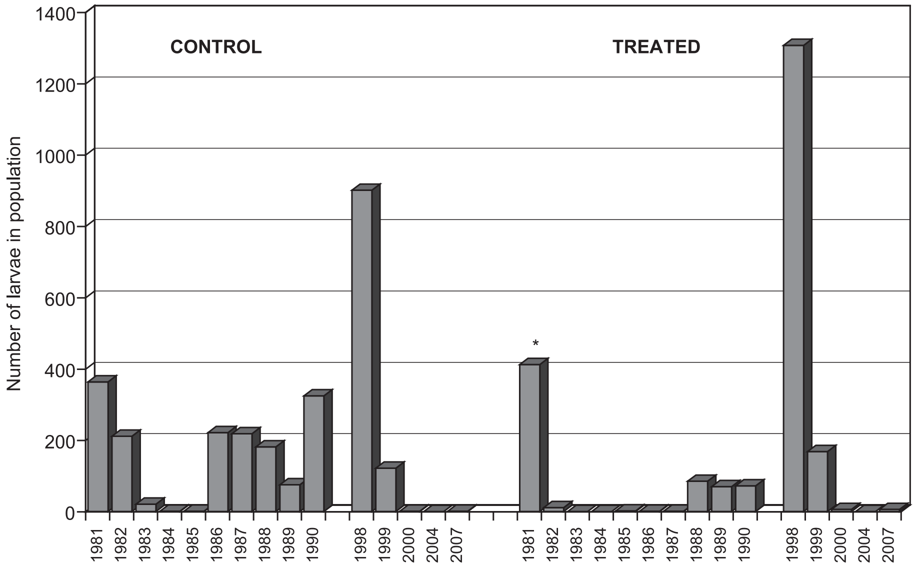

Fig. 2. Population dynamics of control and treated with LesaMNPV populations of the satin moth (*L. salicis*) – "Pobiedziska", Sector II (*year of treatment)

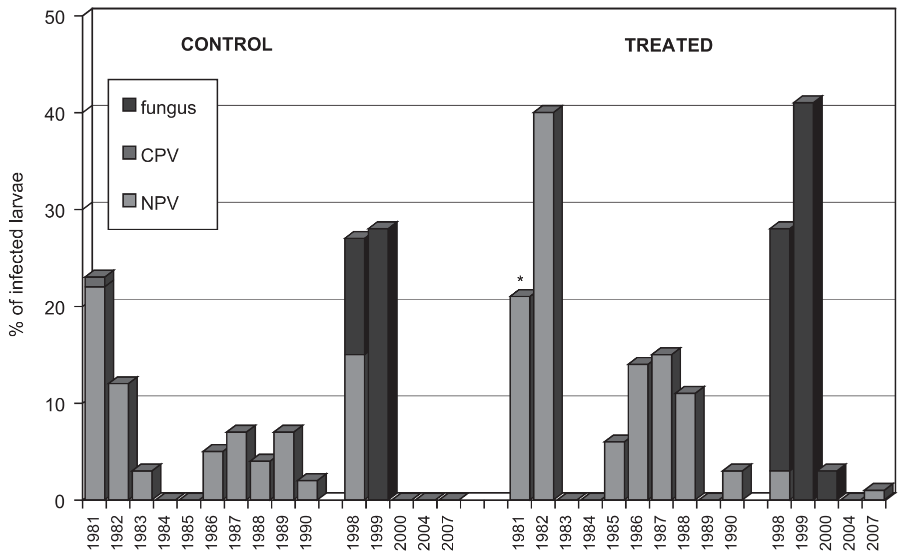

Fig. 3. Healthiness of control and treated with LesaMNPV populations of the satin moth (*L. salicis*) – "Pobiedziska", Sector II (*year of treatment) fungus – Beauveria bassiana; CPV – cypovirus; NPV – nucleopolyhedrovirus

Table 2. Reproduction potential and healthiness of the satin moth (*L. salicis*) treated with nucleopolyhedrovirus LesaMNPV (Pobiedziska 1981)

	Sector	Number of egg masses/ number of females	Number of egg masses/ one female	Average number of eggs/egg mass	Total number of eggs	Number of hatched larvae (%)	% of larvae with virosis
Control	II	24 / 28	0.85	131.1 ± 26.6*	3146	2548 (81.0)	9.0**
	IV	31 / 28	1.10	128.3 ± 35.5	3977	3010 (75.7)	7.0**
Treated	II	5 / 25	0.20	82.8 ± 31.9	414	0 (0.0)	–
	IV	9 / 24	0.37	91.7 ± 25.3	825	7 (0.85)	28.6***

* mean ± standard deviation
** 100 larvae diagnosed
*** all hatched larvae diagnosed

The effects of induced epizootic were detectable for many years (Fig. 2, 4). In both experimental Sectors treated with LesaMNPV pest population remained in the decline phase for subsequent 6 years (1982–1987) of observation. In the first year after induction of epizootic (1982) the spring-summer population numbers in Sector II decreased 40 times when compared with the numbers from the previous year, and in Sector IV occurrence of the satin moth was not observed. During next 3 years (1988–1990) population numbers in both Sectors started increasing, however the outbreak peak observed in the year 1981 was not reached. In the control part of the Sectors (Fig. 2, 4) a slow population decline was observed in the years 1981–1984, and in the period of 1986–1990, satin moth outbreak in control parts of Sector II and IV was observed for 5 and 4 years, respectively. During the whole period of observation nucleopolyhedrovirus was dominant both in treated and control parts of experimental sectors, which depending on the inoculum size regulated comparatively efficiently pest population numbers. It seems that during next 7 years with no monitoring an expanding density of the satin moth resulted in subsequent outbreak whose peak was observed in 1998 (Fig. 2, 4). This outbreak was the biggest mass occurrence of the satin moth in this area during the entire period of observation. A rapid collapse of population numbers observed in the following year (1999) originated from natural epizootic caused by fungus *Beauveria bassiana* (Bals.) Vuill. (26–69% of infected insects). During following years both the satin moth population numbers and virus levels remained at a decline level until 2007 which was the last year of observation (Fig. 2, 3, 4, 5).

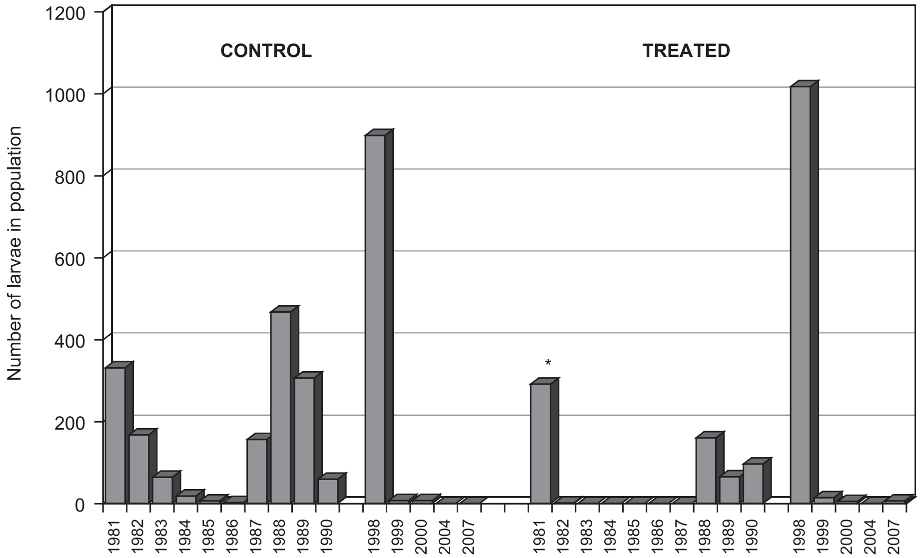

Fig. 4. Population dynamics of control and treated with LesaMNPV populations of the satin moth (*L. salicis*) – "Pobiedziska" Sector IV (*year of treatment)

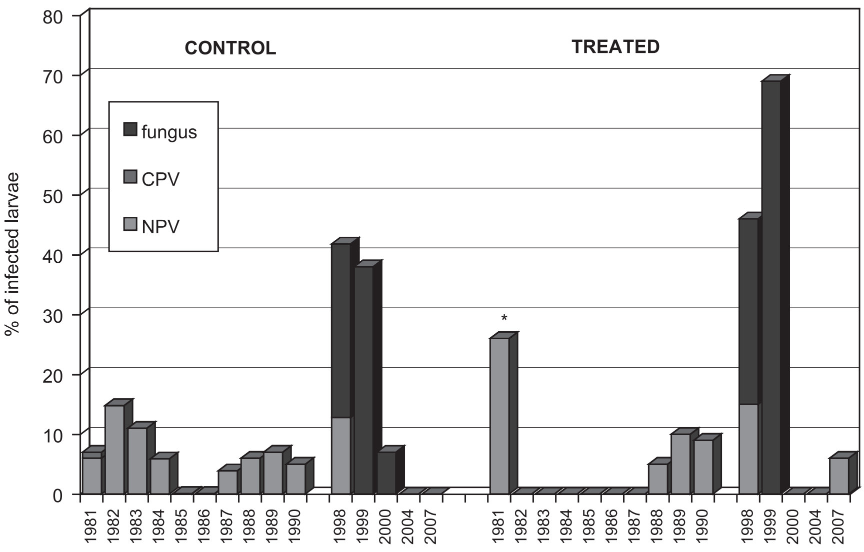

Fig. 5. Healthiness of control and treated with LesaMNPV populations of the satin moth (*L. salicis*) – "Pobiedziska", Sector IV (*year of treatment) fungus – Beauveria bassiana; CPV – cypovirus; NPV – nucleopolyhedrovirus

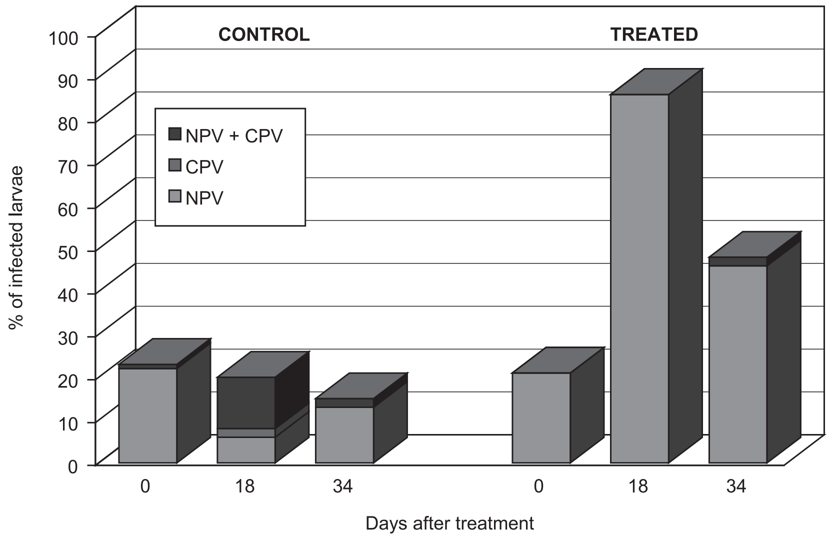

Fig. 6. Virosis expansion in populations of the satin moth (*L. salicis*) in control and treated with LesaMNPV poplars – "Pobiedziska" Sector II. 28.05.1981 CPV – cypovirus; NPV – nucleopolyhedrovirus

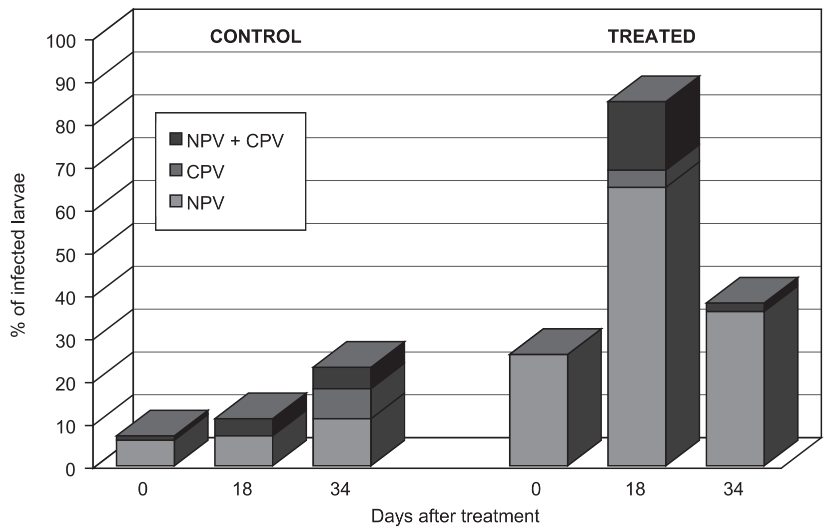

Fig. 7. Virosis expansion in populations of the satin moth (*L. salicis*) in control and treated with LesaMNPV poplars – "Pobiedziska" Sector IV. 28.05.1981 CPV – cypovirus; NPV – nucleopolyhedrovirus

DISCUSSION

Artificially induced epizootics have been one of frequently applied practices in biological control of plant insect pests. A success of this method depends mainly on density and healthiness of controlled pest population, age and virus sensitivity of treated insects as well as virulence of an applied virus strain.

Absence or occurrence of viruses in insect populations as well as a virus level can decrease or increase the efficacy of treatment. Introduction of virus into healthy but sensitive population with low density increases possibilities of virus expansion and results in efficient long-term reduction of pest population numbers. A standard example of achieving such an effect was introduction of a virus from northern Europe into a Canadian population of the European spruce sawfly *Diprion hercyniae* Htg. (Bird 1955). Under natural conditions the majority of populations of many insect species are virus infected to some degree. Natural epizootics observed for years in satin moth populations can be an illustration of such incidents (Ziemnicka 2008). The satin moth due to its ability to relatively quick dispersion (Reeks and Smith 1956) can populate adjacent stands and generate new epizootic centres of cytoplasmic and nuclear polyhedrosis. Population high numbers and a high amount of virus inoculum in population create suitable conditions for epizootic outbreak. Virus epidemic can be accelerated by additional introduction of viruses into insect population, which yet maintains moderate density as it was shown by virus induced epizootic with nucleopolyhedrovirus LesaMNPV in the population "Pobiedziska". There, virus introduction resulted in the epizootic peak only in 8–9 days after the treatment. As stated by Sidor (1967) this period of time is sufficient for complete replication for LesaMNPV virus. Whereas natural satin moth outbreaks when not treated with viruses continued for 2–5 years, the treated insect populations collapsed rapidly within a month. The phase of population decline lasted from 3 to 6 years at the beginning stage of monitoring of the population "Pobiedziska" and for 8 years at the final stage of monitoring. It can be supposed that the extended decline phase observed lately was also a result of fungal epizootic.

Occurrence or absence of cytoplasmic polyhedrosis virus (cypovirus) CPV did not influence progression of virus induced epizootic with LesaMNPV virus. Additionally introduced LesaMNPV virus, even though it was increasing its concentration, did not eliminate cytoplasmic polyhedrosis virus from satin moth population. It can be supposed that nonappearance of antagonistic interaction between both types of viruses observed in investigated population indicates incidence of super-infection. In this case the effects of pathogens' activity are added up followed by rapid and high insect mortality. Interactions of pathogens occur rather frequently in insects since they are deficient in as efficient immune mechanisms as it is observed in vertebrates (Gliński and Jarosz 2000). Synergistic interactions between cytoplasmic and nuclear polyhedrosis viruses in the satin moth can be detected in laboratory conditions (Ziemnicka 1981), whilst in field conditions they are very hard to identify due to technical difficulties with application of many different virus concentrations.

The decline phase of satin moth population that continued for many years after the treatment was a consequence of not only a rapid decrease of the larvae number, but also a decrease of offspring production ability by specimen that survived and were able to undergo metamorphosis. A significant decrease of the number of deposited eggs was observed as well as the low percentage of hatched larvae. This indicates high virulence of the applied virus and its ability to uphold low population numbers also *via* vertical infection.

Application of virus bio-preparation during egg stage would be the most cost-effective timing of satin moth control. Relatively low rates of preparation could promptly secure desired effects of treatments, i.e. efficient protection of cultivated plants by initiation of high pest mortality before it even starts feeding. However, due to hampered diffusion of preparation to egg shell (egg masses are covered with protective white froth) and what's more because of little damage caused by the stage L_1 larvae in natural conditions, control treatments are usually carried out on the spring-summer generation of the stage L_3 larvae and at the beginning of the stage L_4 (Nef 1971; Sidor 1967). During the stage L_4 satin moth larvae increase body weight 6 times in comparison with the weight in the stage L_3 (Ziemnicka 1981). During larval development L_4 is the stage of most intense feeding and of high vulnerability to virus infection, thus timing of this stage is most sufficient for application of treatments. Great amount of eaten leaves mass treated with virus guarantee the inoculum effectual enough for fast replication and attainment of high mortality of treated insects. As shown in the population "Pobiedziska" nucleopolyhedrovirus was not the only epizootic factor for the satin moth. At the same time a robust fungal epizootic was observed in 1998–1999 in tested population. This was the second case of fungal epizootic observed in the region of Wielkopolska, and the first one was recorded in 1994–1996 (Ziemnicka and Sosnowska 1996). In populations of *L. salicis* fungi are pathogens frequently observed, however they are rarely dominant. The observed outburst of fungal epizootic was caused by adequate conditions for its development, i.e. high population density, big amount of fungal inoculum as well as high air temperatures and humidity.

Another important factor critical for successful treatment is selection of sufficient virus concentration. In Belgium, Nef (1971) applied concentration 2×10^9 virus inclusion bodies in 2 liters of water per tree and obtained 93% mortality of satin moth larvae feeding on 10-year-old poplars *P. euroamericana* var. *robusta*. In former Yugoslavia, Kuševska (1972) obtained 100% mortality by application of NPV virus at a rate 2.5×10^3 in 25 liters of spray liquid. Approximately 20-year-old poplar stand attacked by the satin moth consisted of rows of trees with widely expanded crowns. Extensively expanded tree leaves required higher concentration of virus inclusion bodies per tree. Thus, high virus concentration of 4.0×10^{10} inclusion bodies per tree was used and desired efficacy was obtained, however lower rates could be equally efficient.

The results of artificial introduction of nucleopolyhedrovirus LesaMNPV into satin moth population indicated high efficacy of the pathogen in control *L. salicis*. Such treatments could become an important component in plant protection programs for the willow family (*Salicaceae*) in urban areas, parks and alongside roads as well as in sites where chemical treatment would not be advisable.

The results obtained during long-term observation on the effects of virus induced epizootic presented in this study have been reported on for the first time in the subject area.

CONCLUSIONS

1. Artificially introduced LesaMNPV virus into population of the stage L_3 and L_4 larvae of *L. salicis* shows high efficacy both in the peak and early decline phases of satin moth population.
2. LesaMNPV virus once introduced into *L. salicis* population endures for several years that results in continuing reduction of pest population numbers.
3. Epizootic caused by fungus *B. bassiana* (Bals.) Vuill. occurring concurrently with LesaMNPV virus can play an important role in long-term reduction of *L. salicis* population numbers.

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POLISH SUMMARY

EFEKTY INDUKOWANEJ EPIZOOCJI WIRUSOWEJ W POPULACJI BIAŁKI WIERZBÓWKI *LEUCOMA SALICIS* L. (*LEPIDOPTERA: LYMANTRIIDAE*)

Zabieg indukowanej epizoocji wykonano przez wprowadzenie wirusa poliedrozy jądrowej (nukleopoliedrowirusa) *Leucoma salicis* (LesaMNPV) do populacji białki wierzbówki *L. salicis* L. na topoli *Populus nigra* L. Wprowadzenie wirusa (koncentracja 4 x 10^{10} wirusowych wtrętów na drzewo) do populacji owada (stadium L_3–L_4), zarówno w fazie kulminacyjnej jak we wczesnej fazie regresji, powodowało gwałtowne załamanie się gradacji szkodnika. O tempie rozwoju epizoocji decydował stan zdrowotny owadów przed zabiegiem. Populacja o wysokim poziomie poliedrozy jądrowej (około 21% i 26% owadów zakażonych) szczyt epizootyczny osiągała w 18 dniu po zabiegu (85% i 86% owadów z wirozą). Indukowana epizoocja, doprowadziła do stanu depresji populacyjnej trwającej przez okres 6 kolejnych lat. Przypadkowa obecność grzyba *Beauveria bassiana* (Bals.) Vuill. w populacji tego szkodnika w fazie epizootycznej wydłużyła stan depresji populacyjnej *L. salicis* na okres co najmniej 8 lat. Indukowana epizoocja wirusowa powodowała gwałtowne załamanie się gradacji szkodnika i przejście populacji z fazy kulminacji gradacyjnej bezpośrednio w fazę depresyjną z pominięciem fazy regresji, którą obserwowano w populacjach nie poddanych zabiegowi.

Sztucznie wywołana epizoocja wirusowa wpływała na zdrowotność poczwarek i owadów dorosłych, a także na potencjał biologiczny samic i zdrowotność potomstwa. Poczwarki z gąsienic traktowanych preparatem miały mniejszą masę. U poczwarek samiczych wirozę wykrywano częściej aniżeli u poczwarek samczych. Wyrażało się to 6–28-krotnym spadkiem liczby złóż jajowych i 3.5–5-krotnym spadkiem liczby jaj złożonych przez samicę. Potomstwo zakażonych rodziców było 70–800--krotnie mniej liczne w porównaniu z potomstwem rodziców nie traktowanych wirusem i niejednokrotnie (15–28%) wykazywało objawy wirozy.

Indukowana epizoocja, przedstawiona w niniejszej pracy, była pierwszą próbą wieloletniej oceny skuteczności nukleopoliedrowirusa w warunkach naturalnych.