USING BACULOVIRUS TO RESTORE AND CONSTRUCT STABLE NATURAL AND ANTROPINATED ECOSYSTEM FOR CONTROL OF THE *Hyphantria cunea* Drury

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Abstract

The aim of the present paper is to discuss the results of two years attempt of biological control of H. cunea populations with a baculoviral product. In the report there are also submitted the results of the joint application of the biological preparation Virin ABB-3. The preparation is based on viruses of nuclear polyhedrosesis and granuoses with cumulative and synergetic action. In such context, the problem is connected to large application of baculoviral preparation that have become a reality only by elaboration and organization of production of such biological means, work registered after execution of deep biotechnological researches. The criterion of stable ecological system and the methods restoration and construction were also analyzed. Meanwhile, the experiment in which viruses were used and successfully control of the species H.cunea persistently indicate that the insect viruses play an important role in restoring and constructing stable natural and antropinated ecology system.

Key words: *Hyphantria cunea*, natural and antropinated ecology system, biological control, baculoviral preparation, VG, VPN.

The microbial biopesticide market still only represents about 1% of the sales of chemical pesticides (CPL Consultants., 2010; Marrone., 2007). Factors impeding the establishment of strong MPCA markets are complex (Chandler et al., 2011; Marrone., 2007; Ravensberg, 2011) but include the burdensome costs associated with the registration of commercial products that are aimed at relatively small niche markets (Chandler et al., 2011; Ehlers, 2011).

Baculoviruses only infect insects, are ubiquitous in the environment and are known to be important in the regulation of many insect populations. Baculoviruses are host specific, infecting only one or a few closely-related species, helping to make them good candidates for management of crop and forest insect pests with minimal off-target impacts (Hewson et al., 2011). In fact, baculoviruses have been recognized as being amongst the safest of pesticides (Black et al., 1997).

Different biopesticides, such as viruses, (bacteria, fungi, etc.), microorganism products, plant derived products have been developed and used across the world (Mazhabi *et al.*, 2011; Islam and Omar,2012).

Baculoviruses microorganisms have shown to be a good tool for insect pest control. Numerous natural baculoviruses microorganisms have been used as biopesticides worldwide as they are naturally organisms.

Therefore, the forest pest control in Moldova in the 21st century must follow the sustainable forest protection strategy. The forest pest control technology which is reasonable, based on ecological principle, and harmonious with the environmental protection is very important to implement the strategy.

The present paper gives information of baculovirus preparats, we discuss how baculovirus evolution, host range determination and pathogenesis have contributed to their inherent safety for non-target organisms including humans. The virus also can accumulate themselves in the environment and the host population, and it can control the pest insects for a long term by forming epidemic disease in the pest insect population through the external environment stimulation. The quite stable food chain relation of plant-pest insect-natural enemy can be gradually established. Thus it can reduce the risk of the pest insect continuous outbreak and realize the persistent control of pest insects.

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MATERIAL AND METHOD

The researches have been realised on the caterpillars of 2-3 ages of the *H. cunea.* In the study, we used the Nuclear Polyhedrosis Virus, selected and identified in the laboratory of the insect viruses.

For the contamination of the laboratory insects, we used the dosed feeding, which contains 10 polyhedrons for each caterpillar. The monitoring of the insects lot and the estimation of the dead caterpillars has been carried out daily, beginning with the 3rd day of the contamination. The caterpillars *H. cunea* were kept under laboratory conditions at 27˚C.

For infection of larvae there was necessary a preliminary preparation of viral suspensions, using for that purpose pure or initial suspensions and applying dosed infection of insects according to the Vago C. procedure (1972) and its different modifications (Ciuhrii M., Volosciuc L, 1990,1991).

During the process of identification and determination of biological activity of baculoviruses there was necessary its purification. At initial phases purification of VPN and VG does not differ substantially. Dead larvae were soaked with the help of a mixer, and the biological mass was mixed with sterile filtered bidistilate through an apron screen.

For purification of VPN were used several methods, for which we have used the modifications of our institute, consisting of the following phases. Filtered viral suspension is centrifuged within 30 min at 1000 rpm in ŢLN-2 centrifuge. The obtained deposition is washed three times with water. The obtained suspension is centrifuged in the gradient of sucrose concentration (70-20%) and is centrifuged at 3000 rpm within 10 min. Zones with concentration of 40-50% were put together and layered in the gradient of 50-60% and after 15 min of centrifugation there was obtained the fraction of SPVC.

For determination of concentration of baculoviral suspensions there were used different methods, especially electronic microscope (Ciuhrii M.,1991.,Volosciuc L. T . 2007). Titration of baculoviruses with the help of quantum microscopy depends on the kind of virus. Thus, if VPN may be examined with all kinds of optical microscopes, because they have relatively big size (0, 5-10 mcm), then VG having much smaller size (0, 01-0,5 mcm), is at the edge of optical microscope resolution, that's why they were mostly treated with the help of electronic microscopes.

For the determination of baculoviral concentration there are used different methods, especially of electronic and optical microscopy (Ciuhrii M.,1991.,Volosciuc L. T . 2000). Titration is carried out with the help of Goreaiev chamber or in the fixed and colored preparations. There were elaborated different methods of determination of biological activity of baculoviruses. At the initial phase viral suspension is titrated, determining its concentration. Then there is prepared a series of successive dilutions with the help of which are infected larvae of the second age (it is rational to use 40 larvae of the same physiological state). After the third day there is determined the morality of larvae by options, and is being prepared the diagram of "dose-effect" relation. For that reason there is applied the method of sample analysis. Then are made some additional calculations, which allow transformation of axis for obtaining of the "dose-effect" relation in the form of straight line, and not in the form of asymmetrical curve. Construction of diagram allows us to determine the logarithm of the viral suspension dose, which ensures the death of 50% of the experimental larvae. Knowing the virus concentration and volume of viral suspension it is easy to determine lethal concentration (CL_{50}).The mathematical treatment was registered on the 15th day after contamination; the statistical treatment was made according to (Dospehov., 1985; GAR. 1963).

RESULTS AND DISCUSSIONS

The results obtained within some multiple experiments carried out with larvae of second and third age of *H.cunea* lead to the conclusion that surviving of insects and baculoviruses has become possible only at obtaining a moderate biological activity. Thus, there were registered substantial results at the examination of biological activity of viral biological mass obtained from larvae which died on different days after infection with viral suspension (*table 1*).

Figure 1 **Damage of lavae - *H. cunea***

Results placed in the above table show the difference between the parameters of biological activity of biological mass obtained on the different years from the infection with baculoviruses. There are not noticed any substantial differences of biological activity in the case of viral suspension with the same concentration (10^7 pol./ml). Analyzing the biological effectiveness of the baculoviruses we obtained a value of 87-92%.

Table 1

Biological activity Virin-ABB-3 for control of the fall webworm moth (*Hyphantria cunea* Drury)

N	Year testing Virin- ABB-3	Virus preparation and biological standard	Treatments, Kg/ha	Biologic efficacy of treatments, %
1	2012	Virin-ABB-3	0,1	92,0
2		Lepidocid	1,0	-
3	2013	Virin-ABB-3	0,1	84,86
4		Lepidocid	1,0	87,26
5	2014	Virin-ABB-3	0,1	87,1
6		Lepidocid	1,0	87,21
7	Media	Virin –ABB-3		87,0

In the terms of that aspect, biological mass obtained from dead larvae after these days is characterized by parameters specific to wild strains obtained from natural conditions, that aspect induces the difference of biological activity of biological mass obtained from dead larvae on different years of infection and denotes the possibility of application baculoviral preparation and will be very useful for the baculovirus treatments managment.

However for early detection of the fall webworm and moth liquidation, and its further settling prevention it is necessary to know the development terms of the pest. For this aim the phenological calendars for the fall webworm moth allows solving other ecological problems, such as prognoses of pairing predictions for pests and entomophagous, precise determination of treatment terms, and number of generation in the given region. If the dynamics of the number of species according to the living conditions is a multifactorial process, then the number of environmental factors, which significantly influences the number of the populations of a species, is always much smaller. However for early detection of the fall webworm and moth liquidation, and its further settling prevention it is necessary to know the development terms of the pest. For this aim the phenological calendars for the fall webworm moth allows solving other ecological problems, such as prognoses of pairing predictions for pests and entomophagous, precise determination of treatment terms, and number of generation in the given region. If the dynamics of the number of species according to the living conditions is a multifactorial process, then the number of environmental factors, which significantly influences the number of the populations of a species, is always much smaller. This situation can be explained by the fact that non-possessing the ability to adapt simultaneously to a large number of factors, the population is forced to organize its vital activity in order to avoid contact with the majority of limiting factors and to depend on a minimum number of factors.

Figura 2 **Butterflies of fall webworm *H.cunea***

Thus, as the main factor influencing the development of the fall webworm moth is temperature, in connection with the fact that the most efficient treatments were carried out against younger larvae ages of the fall webworm moth we made the phenological calendar for the territory of the Republic of Moldova (*table 2*). In order to achieve the calendar, the method of SERGEEV & LEVINA was used.

The calendar is used in this. Let's suppose that the beginning of egg deposition by fall webworm moth in Moldova occurs on the 15th of May, and the average air temperature in May of the current year is 1°C higher than the multiannual norm.

Table 2

Phenological calendar rendering the beginning of *H.cunea* larvae hatching and the date recommended for the start of protective treatments with Virin ABB-3 depending on the actual dates of oviposition and temperature conditions

Date of the laying beginning	The actual deviation of the average air temperature for a part of the prognosticated period from the multiannual mean, °C													
	0	The terms for carrying treatments	+1	The terms for carrying treatments	+2	The terms for carrying treatments	+3	The terms for carrying treatments	-1	The terms for carrying treatments	-2	The terms for carrying treatments	-3	The terms for carrying treatments
May 1	19	29	16	26	14	24	12	22	21	1.06	25	5.06	30	10.06
May 3	20	30	17	27	16	26	14	24	23	3.06	26	6.06	30	10.06
May 5	21	1.06	18	30	17	31	16	26	24	4.06	27	7.06	31	11.06
May 8	23	3.06	21	1.06	19	1.06	18	30	25	5.06	29	9.06	1.06	12.06
May 10	24	4.06	22	2.06	21	1.06	20	31	26	6.06	30	10.06	2.06	13.06
May 13	26	6.06	24	4.06	23	3.06	23	3.06	28	8.06	31	11.06	4.06	15.06
May 15	27	7.06	26	6.06	25	5.06	25	5.06	30	10.06	1.06	12.06	5.06	16.06
July 10	20	27	19	26	19	26	18	25	20	27	20	27	21	28
July 13	22	29	22	29	22	29	21	28	23	30	23	30	24	31
July 15	24	31	24	31	24	31	23	30	25	20	25	20	26	2.08
July 18	27	3.08	27	3.08	27	3.08	26	2.08	28	4.08	28	4.08	29	5.08
July 20	29	5.08	29	5.08	29	5.08	28	4.08	30	5.08	30	5.08	31	6.08
July 23	1.08	7.08	1.08	7.08	1.08	7.08	31	6.08	208	9.08	2.08	9.08	3.08	10.08
July 25	3.08	10.08	3.08	10.08	3.08	10.08	2.08	9.08	4.08	10.08	4.08	10.08	5.08	11.08
July 28	6.08	12.08	6.08	12.08	6.08	12.08	5.08	11.08	7.08	13.08	7.08	13.08	8.08	14.08
July 30	8.08	14.08	8.08	14.08	8.08	14.08	7.08	13.08	9.08	15.08	9.08	15.08	10.08	16.08

Then at crossing of the first column of the 15th of May and of the column + 1°C we find the date of the appearance of the first larvae generation, namely the 26th of May. Protecţia plantelor impotriva Omizii paroase a dudului este mai eficientă atunci cand larvele sunt de varste mici. At an average deviation of the air temperature for the same period of time by 1 degree, the beginning of the emergence of fall webworm moth larvae will take place on the 30th of May. Normally the larvae do not emerge simultaneously from all laid eggs. The emergence process lasts for another 1-1.5 weeks. Plant protection against *H. cunea* larvae is the most efficient when the larvae are of younger ages. That is why the treatments should be carried out when the larvae emergence from all deposition is finished.

During this period a small part of larvae are at the third, the largest part at the second, and a part at the first stage. Using the presented calendar, it is possible to predict beforehand the terms for beginning protection treatments, that allows not only to prepare the necessary facilities and stock in due time but also to prepare the local human population. By the same terms it is possible to begin cutting out the caterpillars found within the web feeding in the tree crown. Carrying out this work earlier than this term can lead to the case when a part of the nests will be not observed and destroyed. In consequence, the phenological calendar offers the possibility to precisely and the most advantageously use the protection means against the fall webworm moth, and to carry out the mechanical control by nest cutting out before the elder age larvae are settled.

CONCLUSIONS

The insect virus pesticide is only one method to construct and restore the stable ecosystem, other methods can also be applied as the effective methods as long as they are useful to the control of target pest insects and do not cause destruction of the environment.

From the results it can be concluded that this method of fighting, in addition doses viral preparation, special importance has and choosing the most favorable treatment times, about larval development and overgrowth of foliage trees. Treatments should be applied only during the period when larval are the first two age and leafy trees. The method put forward in this paper is the preliminary result of the experiments we have been trying to control the foliage feeding insects for many years. And this method taking the virus as the main measure to restore and construct the stable ecosystem were the pest insects had occurred. The researches point out *H. cunea* critical stage and will be very useful for the baculovirus treatments management.

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