

APPLICATIONS AND PERSPECTIVES OF USING BACULOVIRUSES FOR SUSTAINABLE PLANT PROTECTION

AURELIA STÎNGACI*

ABSTRACT

Păstrarea durabilă a culturilor de microorganismе reprezintă un factor important pentru utilizarea lor atât în calitate de obiecte ale cercetărilor științifice fundamentale, cât și în calitate de obiecte cu potențial biotehologic. Articolul este dedicat control biologic al populațiilor de *Hyphantria cunea* Drury cu un produs baculoviral Virin ABB-3. Aplicarea baculovirusurilor în protecția culturilor deține o promisiune semnificativă în gestionarea insectelor prin livrarea controlată și orientată a produselor biologice. Baculovirusurile au avantaje față de metodele chimice convenționale care sunt asociate cu toxicitatea ecologică. Această revizuire este axată pe aplicarea ecologică ale baculovirusurilor în protecția culturilor agricole, decorative și forestiere.

Key words: *Hyphantria cunea* Drury, Virin ABB-3, baculoviruses, VG, VPN.

Introduction

Biopesticides have attracted attention in pest management in recent decades, and have long been promoted as prospective alternatives to synthetic pesticides. Biopesticides have also attracted great interest in the international research community, with a significant increase in the number of publications devoted to the subject. Chemical use in the parks from Republic of Moldova leads to environmental condition worsening. But due to the large-scale application of broad-spectrum insecticides, *H.cunea* has already developed some level of resistance in Republic of Moldova. Furthermore, frequent applications of pyrethroids and neonicotinoids may cause severe negative effects on organisms that provide ecosystem services including pollination and natural pest control [4].

The legal basis for plant protection in Republic of Moldova and Europe focuses on an Integrated Pest Management (IPM) strategy, that should limit the application of chemical plant protection products to a necessary extent by using a combination of procedures which are under the primary consideration of plant breeding and cultivation measures as well as biotechnical and biological methods (PflSchG 2012). Biopesticides are natural materials derived from animals, plants, and bacteria, as well as certain minerals, that are used for pest control [5]. Currently, biopesticides comprise a small share of the total crop protection market globally, with a value of about \$3 billion worldwide, accounting for just 5% of the total crop protection market [6, 7]. In the United States (US) market, more than 200 products are available, compared to 60 analogous products in the European

Union (EU) market [6]. Because of this pest characterisation, efficient pest management is required. Preparations based on baculoviruses are eco-friendly and specifically effective against lepidopteran larvae [1].

The collapse of insect outbreaks in some species is associated with epizootics of baculovirus disease suggesting that these pathogens can regulate insect populations. In several cases these viruses have been developed for use as biological insecticides for the control of pests of forests and agricultural crops, or as expression vectors in biotechnological applications [2,3]. In the present review we examine the evidence for covert infection of insects by baculoviruses, the similarities between baculoviruses and persistent virus infections in vertebrates, the relationship between virulence and transmission strategy, and the role of covert infection in the ecology of baculoviruses and their hosts-insect pathogenic viruses are a fruitful source of microbial control agents, particularly for the control of lepidopteran pests. Most research is focused on the baculoviruses, important pathogens of some globally important pests for which control has become difficult due to either pesticide resistance or pressure to reduce pesticide residues. Baculoviruses are accepted as safe, readily mass produced, highly pathogenic and easily formulated and applied control agents. New baculovirus products are appearing in many countries and gaining an increased market share.

Mass production of baculoviruses at a cost most potential users can bear remains a significant issue. Production of commercial baculoviruses insecticides is still dependent on in vivo systems utilizing specially reared or wild collected insects

*The Institute of Genetics, Physiology and Plant Protection of Republic Moldova, Chișinău, e-mail:aurelia.stingaci@gmail.com

[7]. In vivo systems for production of baculoviruses in live larvae remain the normal production method for commercial companies and for public sector programs but the relatively high cost of producing baculoviruses in living insects compared to their chemical insecticide counterparts remains a constraint as farm prices are difficult to reduce below \$20 per ha and scaling up in vivo baculoviruses production with its demands for high quality disease-free insects is also a challenge [2]. The use of automation and mechanization in inoculation, rearing, and harvesting has facilitated mass production and made baculoviruses a viable commercial option for the current range and usage scale.

However, the absence of a practical in vivo mass production system, generally higher production costs, limited post application persistence, slow rate of kill and high host specificity currently contributes to restricted use in pest control. Overcoming these limitations are key research areas for which progress could open up use of insect viruses to much larger markets.

The objective of the study was aimed to highlight new agents for biological control of insects with baculoviruses. This study investigated the potential of baculoviruses isolates obtained in IGFP in Republic of Moldova for the control of *Hyphantria cunea* Dr., a dangerous invasion pest which has quickly settled in the territory of the Republic of Moldova during the last three years. This study aimed to highlight new agents for biological control of invasive pest on local baculoviruses strains. The results of the present study revealed the larvicidal potential of baculoviruses isolates found in the larvae of *H.cunea*, local production of biopesticides, which will reduce the final cost of the product and will be more accessible to farmer. This study was addressed to find an environmentally and user friendly, effective control method, which is commercially available and do not require permitting or testing for introduction.

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.

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 bidistillate 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 TLN-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 5000 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 [3, 8]. 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 [3]. Titration is carried out with the help of Goreaiev chamber or in the fixed and coloured 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 mortality 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 [6, 7].

Results and discussions

Reproduction of baculoviruses on the basis of plant feeder insects remains the main way of insect production. That was confirmed by the researches carried out in different scientific and production centres. Baculoviruses adopt mixed-mode transmission strategies that depend on the relative fitness gains that accrue through vertical and horizontal transmission (Figural1).

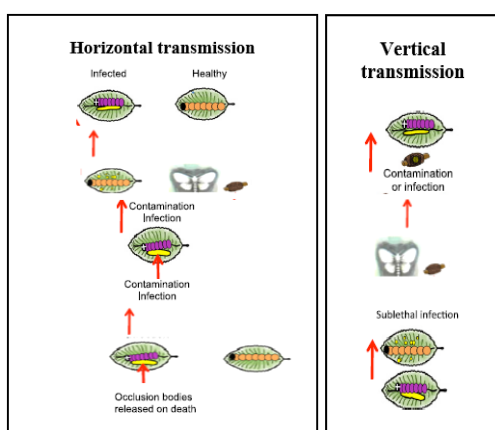


Fig. 1 - Vertical and horizontal transmission of Fall webworm (*Hyphantria cunea* Drury)

These pathogens adopt a mixed-mode transmission strategy involving both horizontal and vertical transmission that is common across a broad range of viruses, parasites, symbionts, and microbiota. Horizontal transmission is usually risky if susceptible hosts are rare, while vertical transmission is safer, but is constrained by host survival and reproductive success. As a result, horizontal transmission is selectively advantageous at high host densities, whereas vertical transmission is favoured in low density host populations. In the case of baculoviruses, these transmission strategies generally exclude one another because the production of massive numbers of OBs for

horizontal transmission results in host death prior to the adult stage.

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 polyhedral /ml). Analysing 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 management.

Results having been analyzed, we may observe, that reduction of attack in comparison with witness in the chemical variant Actara 25WG at the 9 day was 93,9%, but the degree of attack in the biological variant with Virin-ABB-3 at a rate of consume 0,15 kg/ha constituted 89,9%.

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 management.

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. Climatic conditions have influenced negatively development of the culture and the same time the fast development of the pest *Hyphantria cunea*. The preparation Virin-ABB-3 does not negatively act on physiology development of the culture and on formation of plants. Testing of the preparation Virin-ABB-3 at a rate of 0,15 kg/ha for control development, II generation, on plants has demonstrated a significant biological efficacy of 89,9% in comparison with that of chemical etalon Actara of 92,6%.

Acknowledgements

The author is deeply obliged to Dr. Ciuhrii Mircea and Dr. Volosciuc Leonid for their extraordinarily kind and multilateral support.

References

1. BAILEY, A., CHANDLER, D., GRANT, W.P., GREAVES, J., PRINCE, G., TATCHELL, M., 2010 - Biopesticides: Pest Management and Regulation, *CABI International*, Wallingford, p. 232.
2. BAILEY, N. W., MARIE – ORLEACH, L., MOORE, A.J., 2017 - Indirect genetic effects in behavioural ecology: does behaviour play a special role in evolution? *Behav Ecol* doi:10.1093/beheco/ax127
3. CIUHRII, M., VOLOSCIUC, L., SEGELISCAIA, E., 1991 - Activnosti baculovirosov, videlenih iz razni populatii

americanscoi beloi babocki. Edit. Știința. Chisinev., p. 97 [In Russian].

4. EASAC., 2015 - Ecosystem services, agriculture and neonicotinoids. Report No 26. Printed by DVZ-Daten-Service GmbH, Halle/Saale, Germany, <http://web: www.easac.eu> [21.11. 2019]

5. GLARE, T. R., CARADUS, J., GELERNTER, W., JACKSON, T., KEYHANI, N., KOHL, J., MARRONE, P., MORIN, L., STEWART, A., 2012 - *Have biopesticides come of age?* *Trends Biotechnol*, 30, p. 250–258.

6. ДОСПЕХОВ, Б.А., 1979 - Методика полевого опыта. М.: Колос, p. 416

7. ГАП, К.А., 1963 - Методы испытания токсичности и эффективности инсектицидов. М, p. 283.